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Physiological Aspects of Broomrape (*Orobanche* spp.) Parasitism, Host Specificity and Selective Control by Glyphosate.

by

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(ABSTRACT)

In greenhouse experiments, three species of broomrape, *O. aegyptiaca* Pers., *O. ramosa* L., and *O. crenata* Forsk., showed potential to parasitize tomato (*Lycopersicon esculentum* Mill.), tobacco (*Nicotiana tabacum* L.), peanut (*Arachis hypogaea* L.), alfalfa (*Medicago sativa* L.), and soybean (*Glycine max* L.), five major broadleaf crops of Virginia. Peanut was most susceptible, whereas soybean was most resistant to parasitism by broomrape. *O. aegyptiaca* and *O. ramosa* had a wider host range affecting plants in both the Solanaceae and Fabaceae. *O. crenata* affected mainly plants belonging to Fabaceae, with the exception of a single infection of *O. crenata* on tobacco.

In laboratory experiments, two synthetic analogs of strigol, GR 7 and GR 24, showed excellent potential to induce 'suicidal germination' of broomrape seeds. Ethylene (currently used for inducing 'suicidal germination' of *Striga* spp.) and gibberellic acid proved less effective than the strigol analogs in inducing broomrape seed germination. Preliminary investigations on the mechanism of action of the strigol analogs indicated that these compounds induce germination in broomrape seeds possibly by triggering endogenous gibberellin synthesis.

Glyphosate [*N*-(phosphonomethyl) glycine], a foliarly applied, broad spectrum herbicide, when applied to tomato foliage at 75 g/ha was effective in controlling broomrape, but caused injury to the host plants. Single or multiple applications of the herbicide at lower rates (25 or 37.5 g/ha) were ineffective in reducing broomrape parasitism on tomato plants. 2,4-DB [4-(2,4-dichlorophenoxy) butyric acid] at 0.1 kg/ha or higher reduced the number of

broomrape infections on peanut without harming the crop plants. Application of ^{14}C-glyphosate to tomato foliage resulted in translocation of the radiolabel to all parts of the host plant and to attached broomrape shoots. Interestingly, in tomato plants scarcely infected with broomrape, significant amount of the radiolabel translocated from treated leaves to the apical meristem of the host. In tomato plants severely infected with broomrape, however, a major portion of the radiolabel translocated from treated tomato leaves accumulated in broomrape shoots, with very little going to the apical meristem of the host plant. There was no evidence of metabolism of glyphosate in any part of the host or the broomrape. These observations confirmed that glyphosate is translocated from the host foliage to broomrape shoots, where it accumulates in concentrations higher than in the apical meristem of the host. The stronger demand for glyphosate by broomrape shoots than by the apical meristem of the host makes it possible to achieve selective control of the parasite in crops by the herbicide. Proper timing of glyphosate application, however, may be necessary to avoid injury to crop plants from the herbicide. Glyphosate at concentrations as low as 10 mM inhibited germination of broomrape seeds in the laboratory.

Fertilization of the potting medium with nitrogenous compounds prevented parasitism of tomato plants by broomrape. Ammonium sulfate and urea were inhibitory to germination of *O. aegyptiaca* seeds. Nitrate nitrogen did not affect broomrape seed germination. These results indicated that inhibition of broomrape parasitism on crop plants in the presence of nitrogen fertilizers was not due to the effect of the nutrient on broomrape seed germination, but was probably due to an effect on the physiology of the host plants. An integrated approach using synthetic germination stimulants to induce 'suicidal germination', chemicals for selective control of broomrape after its attachment to crop plants and proper fertilization of the soil could provide an effective control of the parasite in crops.

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Table of Contents

1.0 INTRODUCTION **1**

2.0 LITERATURE REVIEW **4**

2.1 GEOGRAPHIC DISTRIBUTION 5

2.2 GROWTH AND DEVELOPMENT OF BROOMRAPE 8

2.3 METHODS OF BROOMRAPE CONTROL 10

2.3.1 Cultural and Mechanical Control Methods 10

2.3.2 Biological Control Methods 11

2.3.3 Chemical Control Methods 12

2.3.3.1 Control of Broomrape Prior to Attachment to Host Roots 13

2.3.3.2 Control of Broomrape After Attachment to Host Roots 20

2.4 LITERATURE CITED 23

3.0 POTENTIAL OF THREE BROOMRAPES (Orobanche spp.) TO PARASITIZE FIVE MAJOR BROADLEAF CROPS **31**

3.1 INTRODUCTION 31

3.2 MATERIALS AND METHODS 34

3.2.1 Influence of Potting Media on Parasitism of Tomato Plants by Broomrape 34
3.2.2 Parasitism of Various Crops with Three Species of Broomrape 35
3.3 RESULTS AND DISCUSSION 36
3.3.1 Influence of Potting Media on Broomrape Parasitism 36
3.3.2 Parasitism of Five Crops with Three Species of Broomrape 39
3.4 LITERATURE CITED 46

4.0 SOME FACTORS AFFECTING BROOMRAPE SEED GERMINATION 47
4.1 INTRODUCTION 47
4.2 MATERIALS AND METHODS 48
4.2.1 Source of Broomrape Seeds 48
4.2.2 Surface Sterilization of Broomrape Seeds 49
4.2.3 Methods of Germinating Broomrape Seeds 50
4.2.4 Effect of Strigol Analogs, Light, and pH on Broomrape Seed Germination 51
4.3 RESULTS AND DISCUSSION 52
4.3.1 Surface Sterilization of Broomrape Seeds 52
4.3.2 Methods of Broomrape Seed Germination 55
4.3.3 Effect of Strigol Analogs, Light, and pH on Broomrape Seed Germination 57
4.4 LITERATURE CITED 67

5.0 EFFECT OF STRIGOL ANALOGS AND OTHER GROWTH REGULATORS ON GERMINATION OF BROOMRAPE SEEDS 69
5.1 INTRODUCTION 69
5.2 MATERIALS AND METHODS 71
5.2.1 Standard Germination Procedure 71
5.2.2 Effect of Ethephon on Broomrape Seed Germination 72
5.2.3 Effect of Gibberellic Acid and Ancymidol on Broomrape Seed Germination 72
5.3 RESULTS AND DISCUSSION 73

5.3.1 Effect of Ethephon on Broomrape Seed Germination 73
5.3.2 Effect of Gibberellic Acid and Ancymidol on Broomrape Seed Germination 75
5.4 LITERATURE CITED 83

6.0 SELECTIVE CONTROL OF BROOMRAPE IN CROPS BY FOLIARLY APPLIED HERBICIDES 85
6.1 INTRODUCTION 85
6.2 MATERIALS AND METHODS 87
6.2.1 Efficacy of Glyphosate and 2,4-DB for Broomrape Control 87
6.2.2 Translocation and Metabolism of Glyphosate 88
6.2.2.1 Plant Material 88
6.2.2.2 Herbicide Application Procedure 89
6.2.2.3 Herbicide Detection and Identification Procedure 89
6.2.3 Effect of Glyphosate on Germination of Broomrape Seeds 91
6.3 RESULTS AND DISCUSSION 91
6.3.1 Efficacy of Glyphosate and 2,4-DB for Selective Control of Broomrape in Crops . 91
6.3.2 Translocation and Metabolism of Glyphosate 101
6.3.3 Effect of Glyphosate on Seed Germination of Broomrape 106
6.4 LITERATURE CITED 113

7.0 INFLUENCE OF VARIOUS NUTRIENTS AND STRIGOL ANALOGS ON GERMINATION AND PARASITISM OF BROOMRAPE 116
7.1 INTRODUCTION 116
7.2 MATERIALS AND METHODS 117
7.2.1 Greenhouse Experiments 117
7.2.2 Laboratory Experiments 118
7.3 RESULTS AND DISCUSSION 119
7.3.1 Effect of Nutrients on Parasitism of Tomato Plants by Broomrape 119

7.3.2 Effect of Various Nutrients on Germination of Broomrape Seeds 122
7.4 LITERATURE CITED 126

8.0 SUMMARY AND CONCLUSIONS 128

Vita 131

1.0 List of Tables

Table 2. 1. Common and chemical names of compounds listed in the literature review. 14

Table 3. 1. Effect of four potting media on growth of 'Rutgers' tomato plants. 37

Table 3. 2. Effect of four potting media on the growth of *O. aegyptiaca* plants parasitizing 'Rutgers' tomato plants. 38

Table 3. 3. Effect of *O. aegyptiaca* parasitism on growth of 'Rutgers' tomato plants. ... 40

Table 3. 4. Parasitism of five crops with three species of broomrape. 42

Table 4. 1. Effect of GR 7 and GR 24 using four different surface sterilization methods on *O. aegyptiaca* seed germination. 53

Table 4. 2. Germination of *O. aegyptiaca* seeds with GR 7 and GR 24 using three different methods. 56

Table 4. 3. Effect of GR 7 and GR 24 on the germination of three species of broomrape. 59

Table 4. 4. Time required for maximum germination of *O. aegyptiaca* seeds after treatment with strigol anologs. 60

Table 4. 5. Effect of light and growth regulators on germination of *O. aegyptiaca* seeds. 62

Table 4. 6. Effect of pH on preconditioning of *O. aegyptiaca* seeds. 64

Table 4. 7. Effect of pH on preconditioning or postconditioning solutions on germination of *O. aegyptiaca* seeds. 65

Table 5. 1. Effect of ethephon and GR 24 on germination of *O. aegyptiaca* seeds 74

Table 5. 2. Effect of gibberellic acid on germination of *O. aegyptiaca* seeds. 76

Table 5. 3. Effect of ancymidol on germination simulation of *O. aegyptiaca* seeds by gibberellic acid and GR 24. 79

Table 5. 4. Effect of ancymidol on germination simulation of *O. aegyptiaca* seeds by gibberellic acid and GR 24. 81

Table 6. 1. Effect of low rates of glyphosate on growth of 'Rutgers' tomato plants. 92

Table 6. 2. Effect of glyphosate on broomrape growth following application of the herbicide to tomato foliage. 94

Table 6. 3. Effect of single and multiple applications of glyphosate on growth of 'Rutgers' tomato plants. 95

Table 6. 4. Effect of glyphosate on growth of broomrape following single and multiple applications of the herbicide on tomato plants. 97

Table 6. 5. Effect of 2,4-DB on growth of peanut plants. 99

Table 6. 6. Effect of 2,4-DB on broomrape growth following application of the herbicide to peanut foliage. 100

Table 6. 7. Distribution of ^{14}C-glyphosate in 'Rutgers' tomato plants infected with broomrape. 105

Table 6. 8. Effect of glyphosate on germination of *O. aegyptiaca* seeds. 109

Table 6. 9. Effect of glyphosate on germination stimulation of *O. aegyptiaca* seeds by GR 7. 110

Table 6.10. Common and chemical names of compounds included in the discussion. . 112

Table 7. 1. Effect of various nutrients on growth of tomato plants. 120

Table 7. 2. Effect of various nutrients on growth of *O. aegyptiaca* parasitizing tomato plants. 121

List of Figures

Figure 2. 1. Broomrape infestations of agricultural importance in the world 6

Figure 2. 2. States in the United States of America in which one or more broomrape infestations have been reported. 7

Figure 2. 3. Structures and molecular weights of strigol, GR 24, and high melting point (HM) and low melting point (LM) isomers of a 3-ring analog of strigol (3-RAS). GR 7 is a mixture of the two 3-RAS isomers. GR in GR 7 and GR 24 refers to the initials of G. Rosebery, the scientist who synthesized the strigol analogs. (Structures adopted from Bradow, 1986). 18

Figure 3. 1. A mature plant of *O. aegyptiaca* parasitizing a tomato plant. 32

Figure 3. 2. (A) *O. aegyptiaca* parasitizing a tobacco plant, (B) *O. aegyptiaca* parasitizing a peanut plant, (C) *O. crenata* parasitizing an alfalfa plant, and (D) *O. crenata* parasitizing a soybean plant. 41

Figure 6. 1. (A) Dry mount and (B) autoradiogram of tomato (cv. Rutgers) plant 3 days after treatment with ^{14}C-glyphosate. 102

Figure 6. 2. (A) Dry mount and (B) autoradiogram of a tomato plant infected with broomrape at an early stage of parasite development 7 days after treatment with ^{14}C-glyphosate. (Note the distribution of radiolabel to the apical meristem of the host plant). 103

Figure 6. 3. (A) Dry mount and (B) autoradiogram of tomato (cv. Rutgers) plant 7 days after treatment with ^{14}C-glyphosate. 104

Figure 6. 4. Thin layer chromatographic analysis of (A) ^{14}C-glyphosate standard and (B) ^{14}C-glyphosate in extracts of broomrape, tomato shoots, and treated tomato leaves. 107

Figure 7. 1. Effect of various nutrients applied during preconditioning on germination of *O. aegyptiaca* seeds. 124

Figure 7. 2. Effect of nitrogenous compounds on germination of *O. aegyptiaca* seeds. . 125

1.0 INTRODUCTION

Broomrapes (*Orobanche* spp.) are devastating root parasites of many economically important crop plants. They belong to the family Orobanchaceae which is characterized by plants having no chlorophyll. Broomrapes, in general, have a wide host range and are most damaging to members of the Solanaceae, Fabaceae, Asteraceae and Brassicaceae families. The most virulent broomrape species are *O. aegyptiaca* Pers. (Egyptian broomrape), *O. ramosa* L. (branched broomrape), *O. crenata* Forsk. (crenate broomrape), and *O. cernua* Loefl. (nodding broomrape). These species occur most commonly in the semi-arid and some temperate regions of the world, *O. aegyptiaca* and *O. crenata* being the most widely spread. Three species of broomrape, *O. ramosa, O. minor* Smith. and *O. ludoviciana* Nutt. occur in the United States. The level of infestation of these species in the country is so limited that no significant economic damage is caused in any crop at present. However, the potential of broomrape to spread and cause damage to many important broadleaf crops is enormous and warrants further investigations.

Several methods of control have been tried against broomrapes, but most have proved ineffective. The only truly effective means of broomrape control at present is soil sterilization, either by chemical fumigation or by solar heating. Soil sterilization, however, is

very expensive and cannot be adopted on a large scale. There is a serious need, therefore, to develop other means of parasitic weed control that are both effective and economical.

One of the major difficulties encountered in effective control of broomrape is the tremendous seed production potential of the parasite. Broomrape seeds are small, and each plant is capable of producing thousands of seeds which become distributed throughout the soil profile. The seeds generally do not germinate unless they are found in the rhizospheres of host or some nonhost plants (trap crops) which exude suitable germination stimulants. The seeds remain viable in the soil for very long periods of time. These characteristics foster the accumulation of vast stores of seeds in the soil, so that crop rotation, as a means of reducing the inoculum in the soil, is not efficient. Controlling broomrape by means of mechanized cultivation is also not feasible since most of the parasite plants appear within crop rows. The development and use of new crop cultivars resistant to broomrape has been limited. Numerous herbicides tried against broomrape also have proved quite ineffective.

Two new approaches show promise in controlling broomrape in crops. The first is aimed at reducing the seed populations of the parasite in the soil and the second approach is directed towards preventing production of new seeds. These approaches include the stimulation of seed germination of broomrape and its control by chemical means after the parasite has established itself on host plants.

Parasite seeds in the dormant state are quite resistant to most control measures except soil sterilization by fumigation or solar heating. Once broomrape seeds have germinated, they require host plants to establish themselves and complete their subsequent development . Thus, it has been recognized that broomrape seeds are most vulnerable to destruction immediately after germination but prior to their establishment on host roots. If broomrape seeds are induced to germinate in the absence of host plants, the young seedlings are unable to support themselves and soon die. The stimulation of parasite seed germination in the absence of host plants is referred to as 'suicidal germination'. Several synthetic analogs of strigol, a chemical isolated from the roots of cotton (*Gossypium hirsutum* L.) and

found to be an effective germination stimulant of witchweed (*Striga* spp.), have proved very effective in stimulating 'suicidal germination' of broomrape seeds.

Chemical control of broomrape after attachment to host roots may be aimed at attacking the parasite directly. This approach has not proved very effective due to lack of a truly selective herbicide that can control the parasite without affecting the crop plants. Alternatively, an indirect approach is being tested by which the herbicide applied to host plants controls the attached broomrape without adversely affecting the crop plants. Glyphosate [*N*-(phosphononmethyl) glycine], a nonselective, postemergence herbicide that is readily translocated from the point of application on the leaves to all parts of the plant, shows promise for selective control of broomrape in some crops.

The objectives of this research were to determine the potential of three of the most devastating species of broomrape to parasitize some major broadleaf crops of Virginia under greenhouse conditions; to investigate the factors affecting the germination of broomrape seeds in the laboratory; to test the efficacy of two synthetic analogs of strigol for inducing 'suicidal germination' and to determine the mechanism involved in germination stimulation of broomrape seeds by these strigol analogs; and to investigate the effect of glyphosate and 2,4-DB on broomrape parasitism on crop plants and the fate of glyphosate in tomato plants infected with broomrape. Some experiments were also conducted to investigate the effect of various nutrients on broomrape parasitism on crop plants in the greenhouse and on broomrape seed germination in the laboratory.

2.0 LITERATURE REVIEW

Broomrapes (*Orobanche* spp.) are phanerogamic holoparasites that subsist on many economically important plants. These belong to the family Orobanchaceae which contains 13 genera and numerous species (125). The genus *Orobanche* alone is estimated to contain as many as 150 species (76). Some broomrape species are host specific while others have a wide host range. Among the latter, most are devastating parasites of economically important plants. *O. aegyptiaca* Pers., for example, infests more than a hundred hosts including some important crops (56). Broomrapes parasitize most commonly the members of Fabaceae, Solanaceae, Asteraceae and other dicot families. They generally do not occur on monocots; however, one report has indicated that *O. cernua* may parasitize wheat (*Triticum aestivum* L.) (cited in 76). The crops that are most seriously affected by these parasites include tomato (*Lycopersicon esculentum* Mill.), broad bean (*Vicia faba* L.), potato (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), carrot (*Daucus carota* L.) and sunflower (*Helianthus annuus* L.).

2.1 GEOGRAPHIC DISTRIBUTION

Broomrapes occur mainly in areas with hot and dry climates (Figure 2.1). The Middle East is widely infested with four of the most virulent species, namely *O. aegyptiaca*, *O. crenata* Forsk., *O. cernua* Loefl., and *O. ramosa* L. (86). They are the most widespread of the flowering parasites in Jordan (1) and frequently destroy broad bean in Egypt (123). Broomrapes cause considerable damage to legumes in the Mediterranean areas (106). They are reported to occur on clovers (*Trifolium* spp.) in Bulgaria (84) Czechoslovakia (66) and Yugoslavia (74). *O. crenata* is a serious pest of broad bean and pea (*Pisum sativum* L.) in Southern Spain (69, 70, 71, 72). *O. ramosa* is a pernicious parasite of many crops in Romania (114). Some species of broomrapes are reported to occur in Central Asia (56). *O. ramosa, O. aegyptiaca* and, less frequently, *O. cumana* Wallr. are serious problems in all tobacco-growing regions of the USSR (112).

Presently, three broomrape species that have the potential to cause damage to broadleaf crops exist in the United States (Figure 2.2). These are *O. ramosa, O. minor* Smith and *O. ludoviciana* Nutt. The former two broomrape species were introduced into the United States from the old world, whereas *O. ludoviciana* is considered to be native to North America (76). *O. ramosa* was first found in the United States in Kentucky in 1890 and later in New Jersey and in a greenhouse on Long Island, New York. Infestations of this parasitic weed reached devastating levels on tomato crops in the Sacramento Valley of California in 1959, which triggered an extensive control program involving fumigation of the soil with methyl bromide to destroy broomrape seeds (119). It is still a recurring problem in that state (79). Recently, a highly virulent strain of *O. ramosa* was discovered in Karnes county, Texas (78, 104). Although this infestation is in a non-cropped area, it showed potential to parasitize tomato, tobacco, sunflower, bell pepper (*Capsicum frutescens* L.), broad bean, celery (*Apium graveolens* L.), and other economically important crops (33, 77). An eradication program is underway against this noxious weed in that state. In a recent herbarium survey, the U.S.

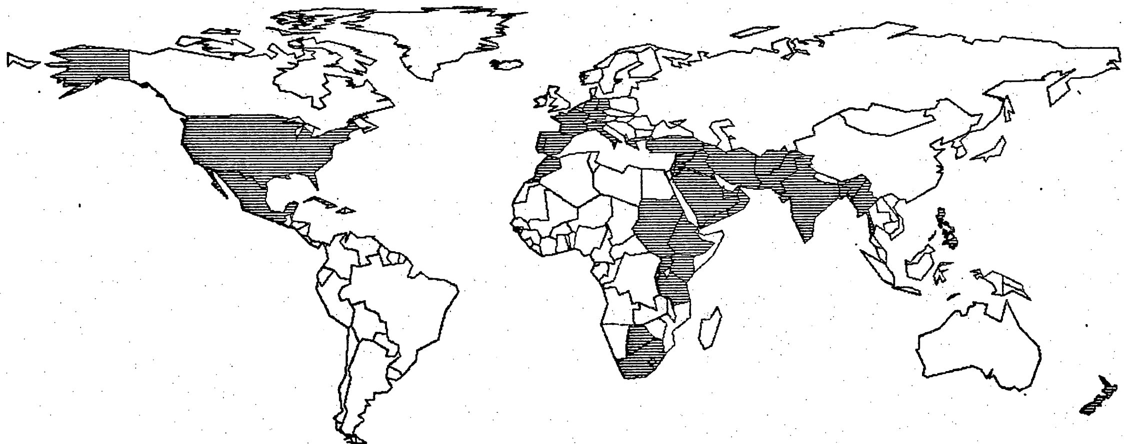
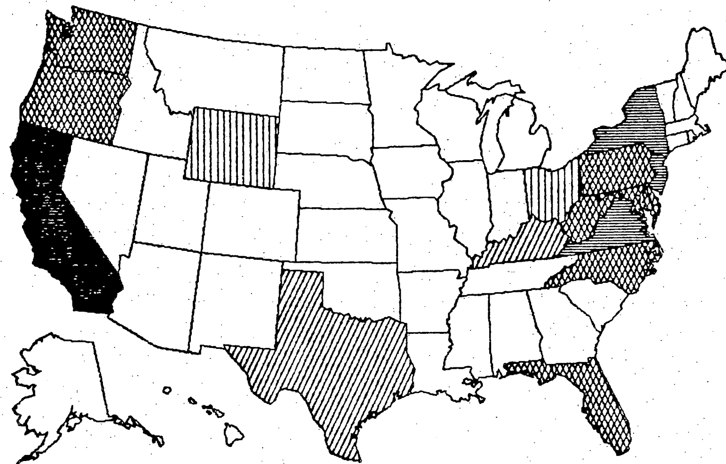


Figure 2. 1. Broomrape infestations of agricultural importance in the world.



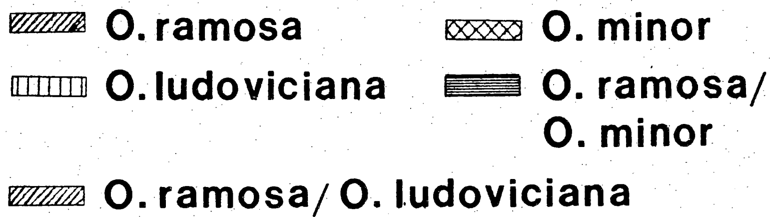


Figure 2. 2. States in the United States of America in which one or more broomrape infestations have been reported.

National Museum, Smithsonian Institution was found to contain one specimen of *O. ramosa*, which was collected from the Blue Ridge Parkway in Virginia in 1951.

O. minor is considered less damaging than *O. ramosa*. It has been found in 10 eastern and two western states, including Virginia, and the District of Columbia (36). It is not considered a major problem on any crop in the United States at present. However, a similar situation existed in New Zealand for many years, after which *O. minor* became a serious problem on clovers (34). It is viewed as a potential threat to the tobacco crop also in that country (53).

O. ludoviciana is a parasite of commercial crops, such as tobacco and tomato in Ohio, Wyoming, and California (17, 85, 111).

2.2 GROWTH AND DEVELOPMENT OF BROOMRAPE

Broomrapes reproduce by means of seeds which are dark brown, oval and very small (about 0.35 mm by 0.25 mm) and can persist in the soil for more than 10 years (57). They consist of a hardened testa surrounding a fatty endosperm, at one end of which is an undifferentiated embryo (57). Each broomrape plant is capable of producing numerous seeds. For example, each *O. crenata* plant can produce up to 500,000 seeds (25).

The process of broomrape seed germination may be divided into two phases. The first is the preconditioning (or pretreatment) phase, during which the seeds undergo conditioning under suitable temperature and moisture. The preconditioning phase may range from several days to a few weeks depending on the species (9, 13, 59). *O. ramosa* seeds, for example, showed better germination when preconditioned for 1 week as compared to seeds preconditioned for 2 or more weeks (5). *O. minor* and *O. crenata* seeds require two weeks of preconditioning before they become receptive to a germination stimulant (28, 29). The second is the germination phase, during which preconditioned broomrape seeds produce a radicle

(also referred to as 'germ tube' or 'procaulome' in the literature on *Orobanche*) in response to a stimulation by a chemical present in the root exudates of host and some nonhost plants. The radicle can grow up to a length of 2 to 3 mm (57).

After emergence, the radicle attaches to host roots mainly in the region of root elongation and absorption. Following attachment to host roots, the radicle produces haustoria that penetrate the host tissue and establish a link with the host's vascular system. The part of the broomrape seedling that remains outside the root tissue gives rise to a tubercle. The tubercle may give rise to secondary haustoria. When the vascular connections are fully established, the tubercle develops into a shoot which emerges above the soil surface and bears flowers. The subterranean tapping of host plant nutrients by broomrape may occur for about 60 days, whereas emergence, flowering and seeding of the parasite may last for only 15 to 18 days (75).

The growth and development of broomrape depends on the stage of development of the host plants. Maximum germination of broomrape seeds usually occurs 10 to 15 days before the host attains its flowering stage (4). Subsequent establishment and development of the parasite on host plants was most favored at the flowering stage of the host (75). Thus, it appears that the most active phase of broomrape parasitism occurs when the crop has almost completed its vegetative phase and has entered the reproductive phase of growth. The maximum damage due to broomrape parasitism, therefore, occurs directly on fruit development and/or seed production of the crop. It has been reported that *O. ramosa* can reduce the yield of tomato by about 30% (23). On sandy soils of tomato-growing areas in Hungary, broomrape can cause up to 75% loss in tomato yield (46). An average of four *O. crenata* plants per broad bean plant can reduce the yield of the crop by half (73). In tobacco, broomrape can drastically reduce leaf yield of the crop. *O. cernua* has been reported to reduce the yield of tobacco by up to 52% depending on the time and intensity of infestation and availability of soil moisture (64, 65).

2.3 *METHODS OF BROOMRAPE CONTROL*

2.3.1 Cultural and Mechanical Control Methods

Several methods have been employed for the control of broomrapes. Hand pulling of broomrape stems has been the most commonly used method to control broomrape and is still practiced in Iraq (38). However, this method is labor intensive and injurious to crop plants. Controlling broomrape by means of mechanized cultivation is also not feasible since most of the parasite plants appear within the crop rows. Burying of broomrape seeds deep in the soil by plowing has also been tested. However, this has not proved effective, because broomrape seeds can remain viable in the soil for more than ten years (57, 96). A recent report has indicated that *O. aegyptiaca* and *O. cernua* seeds can remain viable after passing through the rumen of rams (47).

The observation that seeds of broomrape can germinate not only in the vicinity of roots of host plants, but also in the vicinity of the roots of some nonhost plants (18) suggested the use of these nonhost plants as trap crops to reduce the level of broomrape infestation (8, 12, 58). Chabrolin (19) identified 65 nonhost plant species that can stimulate germination of broomrape seeds. The results of field experiments applying this principle showed varying degrees of success. In an experiment performed in Bulgaria, the incidence of broomrape in tobacco was reduced to a sixth or an eighth of its original level after a preliminary seeding with white mustard (*Sinapsis alba* L.) resulting in an additional yield of 400 to 500 kg/ha of higher quality tobacco (6). In another experiment, planting flax (*Linum usitatissimum* L.) for a period of 4 to 6 weeks prior to planting tomato resulted in considerable reductions in *O. ramosa* infestation (3). Experiments in England, however, resulted in a more limited success. A preliminary sowing of flax did not reduce the incidence of *O. minor* infestation in red clover (*Trifolium pratense* L.) significantly (26). The efficacy of using trap crops to reduce broomrape

infestations is restricted due to the fact that there are enormous amounts of broomrape seeds in an infested soil that are generally dispersed throughout the tilled layer. This results in only a small proportion of the broomrape seeds present in the soil finding the germination stimulus in the rhizosphere of trap crop plants and being destroyed for lack of a suitable host (26, 80).

The nutrient status of the soil has been observed to affect the infestation of broomrape and its parasitism on host plants. Farmers in Jordan have claimed that the addition of manure to soil reduced the infestation of broomrape in their fields (1). High levels of nitrogen reduced *O. ramosa* infestations significantly in tobacco and tomato, but also reduced the yield of tomato (2, 58). However, when nitrogen was applied with potassium and phosphorus, broomrape infestation was drastically reduced with an actual increase in tomato yield (2).

2.3.2 Biological Control Methods

Biological control of broomrape has been attempted mainly in Eastern Europe and USSR by means of the insect *Phytomyza orobanchia* Kalt., which feeds on the inflorescence of the parasite (62, 67, 81, 82, 113). Following initial success in small plots, the control of *O. aegyptiaca* by means of *Phytomyza* species was performed on a large scale in the USSR. Treatment was accomplished by distributing perforated plastic bags containing pupae at the rate of 500 to 1000 per hectare. This resulted in killing more than 50% of the broomrape plants and preventing the rest from setting seed (63).

Besides insect larvae, certain fungi have shown promise for control of broomrape. In small plot experiments, the fungus *Fusarium solani* and the most aggressive *F. oxysporum* effectively controlled *O. ramosa* in tomato at the tubercle stage of the parasite in the first two years of inoculation (46). The fungus showed no deleterious effects on seedlings of watermelon (*Cucumis melo* L.) and tomato (83). In certain soils, *Rhizoctonia solani* has been detected as the main factor in suppressing the growth of branched broomrape in tomato (41). Although some success has been achieved in obtaining biological control of broomrape,

particularly in the USSR, this method alone is unlikely to provide control of parasitic weeds in the near future (40).

2.3.3 Chemical Control Methods

Most of the research efforts in controlling broomrape have been concentrated on finding chemicals that can be used effectively in the field. These chemicals may be intended to attack broomrape directly or indirectly. A direct attack on broomrape may involve preplanting treatment of the soil with chemicals capable of destroying the seed reserve, preventing germination or harming the germinating seeds and young seedlings of the parasite. Chemicals may also be used to stimulate germination of broomrape seeds in noncrop situations so that they are killed for lack of nutrients from a suitable host. This stimulation of broomrape seed germination in the absence of a suitable host is termed 'suicidal germination'. Chemical treatments can also be applied to broomrape after emergence in order to inhibit their further development, and especially to prevent new seed formation.

Indirect control of broomrape may be achieved by treatment of the parasite through the host plant just prior to or after attachment to host roots. Systemic herbicides such as glyphosate can prove most effective in this case. Treatments may also be aimed at impairing the ability of the host plants to exude stimulants for broomrape seed germination. Finally, identification and breeding of host crops such as tomato that are partially resistant to both infection from broomrape and injury from herbicides that are effective on broomrape may assist in dealing with the broomrape problem in the field.

2.3.3.1 *Control of Broomrape Prior to Attachment to Host Roots*

Soil Sterilization: One of the most successful approaches that has been directed towards reducing broomrape seed populations in the soil is soil sterilization by either fumigation with chemicals or solar heating.

Methyl bromide has long been recognized as an effective soil fumigant against soil-borne pathogens and weeds. Hamilton (44) was one of the first to show that methyl bromide, when dissolved in various hydrocarbons such as methyl or ethyl alcohol, could be applied to plants and soil as a liquid insecticide. Soil fumigation with methyl bromide provided effective control of *O. ramosa* in California (119, 120, 121). Methyl bromide at 500 kg/ha or metham sodium at 1500 L/ha provided excellent control of *O. minor* in tobacco. Di-Trapex® and of *O. crenata* in broad bean (122) at 750 L/ha or dazomet granular at 400 kg/ha proved to be less effective than methyl bromide or metham sodium (see Table 2.1 for chemical names). However, leaf quality of tobacco was adversely affected by metham sodium (52). Lowering the dose of metham sodium to 500 L/ha resulted in considerable increase in tobacco yield with no adverse effect on its quality (53).

Ethylene dibromide (EDB) applied alone or in combination with chloropicrin was ineffective against *O. aegyptiaca* or *O. ramosa* subsp. *muteli* but gave effective control of *O. crenata* at 120-480 kg/ha in pea (51). The application of EDB can be carried out simultaneously with sowing of crops such as broad bean or pea, thus reducing labor costs. Use of kerosene as a diluent for EDB can also be eliminated since it did not prove more effective than water plus a wetting agent (88). Methyl bromide and EDB should be applied to the soil when it is slightly moist. Application of these fumigants to very dry or very moist soil are generally less effective. Also, while injecting the fumigants, the area must be covered with plastic in order to trap the gas. Metham sodium should be watered into the soil to a depth of about 20 cm.

Soil fumigation with methyl bromide and other fumigants requires the injection of the chemical into the soil and covering of the treated area for 24 hours with polyethylene sheets. For this reason, soil fumigation is very expensive. In spite of its cost, however, methyl bromide

Table 2. 1. Common and chemical names of compounds listed in the literature review.

Common name	*Chemical name*
Chlorpropham	isopropyl m-chlorocarbanilate
2,4-D	(2,4-dichlorophenoxy) acetic acid
Dalapon	2,2-dichloropropionic acid
Dazomet	tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione
DBCP	1,2-dibromo-3-chloropropane
Dichlobenil	2,6-dichlorobenzonitrile
Dinitramine	N^4,N^4-diethyl-α,α,α-trifluoro- 3,5-dinitrotoluene-2,4-diamine
Dinoseb	2-sec-butyl-4,6-dinitrophenol
Diphenamid	N,N-dimethyl-2,2-diphenylacetamide
Di-Trapex®	dichloropropane + dichloropropene + methyl isothiocyanate
EDB	1,2-dibromoethane
Glyphosate	N-(phosphonomethyl) glycine
Malic hydrazide	1,2-dihydro-3,6-pyridazinedione
Metham sodium	sodium methyldithiocarbamate
Nitralin	4-(methylsulfonyl)-2,6-dinitro-N-N-dipropylaniline
Oryzalin	3,5-dinitro-N^4,N^4-dipropylsulfanilamide
Oxadiazon	2-tert-butyl-4-(2,4-dichloro-5-isopropoxyphenyl)-Δ^2-1,3,4-oxadiazolin-5-one
Pendimethalin	N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine
Pronamide	3,5-dichloro-(N-1,1-dimethyl-2-propynyl)-benzamide
TCA	trichloro acetic acid
Telone®	1,3-dichloropropane
Trifluralin	α,α,α-trifluoro-2,6-dinitro-N,N- dipropyl-p-toluidine

fumigation is considered to be the most reliable method and the most extensively used where economically feasible. Besides controlling broomrape, methyl bromide fumigation leaves the soil sterile controlling soil-borne diseases, nematodes and annual and perennial weeds.

A recent technique of controlling broomrape by solar heating of the soil has shown considerable success (48). This technique may be best suited to small vegetable farms due to its relatively simple handling and additional positive effects against annual weeds and nematodes (11, 60). However, this method also is expensive due to the high cost of polyethylene mulching required.

Chemical Stimulation of Germination: Seeds of broomrape are quite persistent and resistant to most control measures except to soil sterilization by such methods as fumigation and solar heating. In order to control broomrape selectively in crops, broomrape seeds must germinate. Once broomrape seeds germinate, the young seedlings attach themselves to the roots of susceptible crops and become established. However, when broomrape seeds are stimulated to germinate in the absence of host plants (suicidal germination), the parasite seedlings die due to lack of nutrition.

It was first observed in 1887 that broomrape seeds germinated only in the immediate vicinity of a host root (cited in 12). The host root was thought to exude a chemical substance that stimulated the germination of broomrape seeds (80). Subsequently, a dry residue that when redissolved in water stimulated the germination of *O. crenata* seeds was obtained from the roots of a host plant. No spontaneous germination of broomrape seeds was observed in the absence of such a stimulatory substance (19) or the host itself (108).

The predominant factor in root exudates responsible for germination is host specific (108). However, many nonhost plants are capable of stimulating the germination of broomrape seeds (14, 64). Chabrolin (19) showed that *O. crenata* seeds germinated in the presence of chickpea (*Cicer arieticum* L.) or flax root exudates under laboratory conditions. Neither of these plants is a host of broomrape. Moreover, flax root exudates proved more effective in inducing broomrape seed germination than did tomato or tobacco, the normal hosts (7, 43).

Root exudates from flax, corn (*Zea mays* L.) and sorghum (*Sorghum vulgare* L.), which are not parasitized by *O. minor*, also stimulated the parasite seed germination more than those of clover, which is a normal host (16). Root exudates have been detected to occur within 3 days of transplanting of flax and within 15 days of transplanting of tomato, tobacco or sorghum plants (7). Some reports have also suggested that root exudates and solutions obtained from tissues of tomato have only a small or no effect on germination of broomrape seeds (32, 117, 124). Chemical stimulants applied to broomrape seeds adequately preconditioned in the soil are more likely to induce germination than chemicals applied to seeds not fully preconditioned. Mung bean (*Vigna radiata* L.) seedling exudates have proved nearly four times more effective in stimulating germination of preconditioned seeds than unconditioned seeds (20).

Several naturally occurring and synthetic growth regulators and nutrients have been tested for inducing 'suicidal germination' of broomrape seeds. Izard and Hitier (cited in 45) found that pyridoxine and nicotinamide stimulated germination of *O. ramosa* seeds. In 1954, the same authors, realizing the compensatory action of magnesium in certain vitamin-deficient plants, tested the effect of magnesium carbonate and sulfate on the germination of *O. ramosa* seeds and found both to be stimulatory. Their results were confirmed by Donini (27), who worked with *O. crenata* seeds and found that the amino acids, proline and ornithine were also effective. Whether these merely act as precursors of nicotinamide remains undetermined.

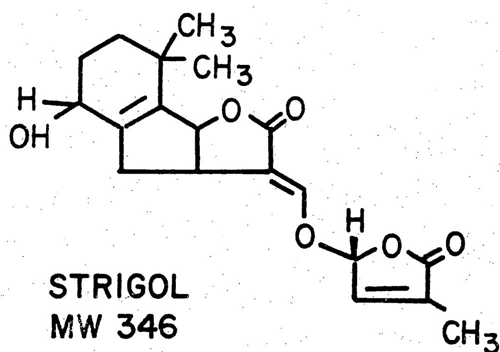
Izard and Hitier (cited in 45) reported that gibberellic acid was effective in inducing germination of *O. ramosa* seeds, and Privat (89) found it stimulatory to *O. hederae* seed germination. Furthermore, Nash and Wilhelm (80) reported that, although *O. ludoviciana* seeds germinated spontaneously, the germination percentage was considerably increased by gibberellic acid, and Abu-Shakra et al. (5) found that *O. ramosa* seeds, pretreated with gibberellic acid gave higher germination than seeds pretreated with water or host root diffusates. Rangaswamy (95) showed that seeds of *O. aegyptiaca* could only germinate in *in vitro* if gibberellic acid, coconut milk or casein hydrolysate were incorporated into the basic

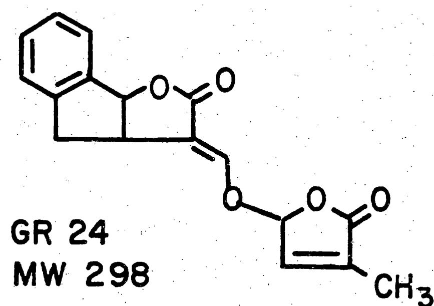
culture medium. Of the three gibberellic acid was most effective. Nash and Wilhelm (80) suggested that, in nature, gibberellins may be exuded in small amounts into the rhizosphere of young plants, and thus may play a part in the germination of broomrape seeds. Auxins (indoleacetic acid and naphthaleneacetic acid) have also been found to stimulate germination of broomrape seeds to some extent (37, 112).

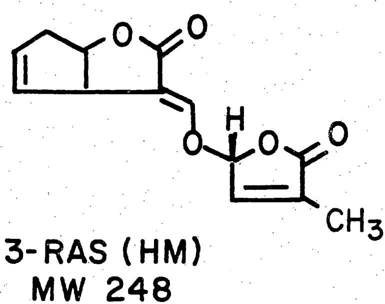
Ethylene is known to stimulate germination of many weed seeds. It has been found to be very effective in stimulating germination of witchweed (*Striga* spp.) seeds. However, this growth regulator was found to have no or only a slight effect in inducing germination of broomrape seeds (20, 30, 58).

In recent years, considerable interest has been generated in identifying and isolating the chemical factors in root exudates of plants capable of stimulating broomrape seed germination. Brown et al. (14, 15, 16) extracted, purified and characterized the stimulant for *O. minor* seeds from charcoal eluates that had been allowed to adsorb the stimulant from root exudates of flax seedlings. Analysis of host root exudates by paper chromatography indicated that the stimulatory fluid consisted of not only germination stimulants but also inhibitors and materials that were inactive by themselves but which acted synergistically to enhance germination (30, 117). This fluid was suggested to contain an acidic or potentially acidic lactone group, possibly similar to 'strigol', a chemical extracted from the roots of cotton and found to stimulate germination of witchweed (14, 68). Subsequently, it was demonstrated that strigol could stimulate the germination of broomrape seeds (55).

The isolation and identification of the structure of strigol led to the synthesis of some strigol analogs or GR compounds (21, 22, 55) (Figure 2.3). These strigol analogs have shown promise for *O. ramosa* (97, 98, 100, 103), *O. crenata* (87) and *O. minor* (109) control *in vitro.* GR 7, which is a mixture of two isomers of a three-ring-analog of strigol, at 3 ppm incorporated into the soil 4 to 6 weeks before transplanting tomato provided 77 to 85% control and a delay of 30 to 40 days in emergence of *O. ramosa* shoots under greenhouse conditions (99). Treatment with this strigol analog proved to be better than flax root exudates in stimulating *O. crenata* seed germination. Exposure of the seeds briefly (5 to 20 min) to calcium







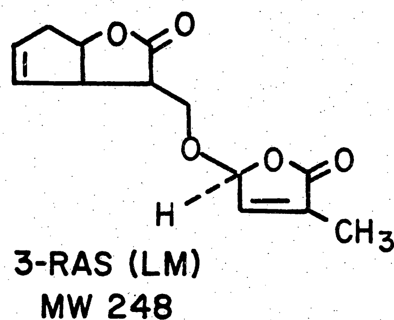


Figure 2. 3. Structures and molecular weights of strigol, GR 24, and high melting point (HM) and low melting point (LM) isomers of a 3-ring analog of strigol (3-RAS). GR 7 is a mixture of the two 3-RAS isomers. GR in GR 7 and GR 24 refers to the initials of G. Rosebery, the scientist who synthesized the strigol analogs. (Structures adopted from Bradow, 1986).

hypochlorite, in addition to GR 7 or flax root exudate, further increased germination. Calcium hypochlorite applied alone was less effective (87). Strigol and strigol analog, GR 45, were shown to stimulate germination of *O. minor* seeds. GR 7, however, was much less effective in stimulating germination of *O. minor* seeds (109). Other strigol analogs, GR 24, GR 28 and GR 41, have also been reported to stimulate germination of *O. ramosa* seeds *in vitro*. Maximum germination with these strigol analogs was obtained at 0.1 to 1 ppm 15 days after incubation of preconditioned broomrape seeds at 25°C. GR 28 was more active than GR 41 when embedded in the soil as a layer or incorporated in the soil (97, 98).

Several chemicals, apart from the growth regulators and strigol analogs, have been found effective in stimulating germination of broomrape seeds. Malic acid + ADP, lipoic acid and betaine stimulated germination of seeds of certain species of broomrape *in vitro*.

Seed Germination Inhibitors: Some plants have been known to produce germination inhibitors that inhibit broomrape seed germination even in the presence of a suitable stimulant. Root exudates from suitable hosts have been observed to inhibit broomrape seed germination when applied in high concentrations (117). Exudates from malic hydrazide treated tobacco plants were found to inhibit germination of broomrape seeds (35). It was thus suggested that host root exudates contained inhibitors besides stimulants that, when applied in high concentrations, inhibited germination (30, 117, 118). Whitney (117) showed that extracts from species which did not trigger germination of *O. crenata* seeds contained compounds which would inhibit germination and multiply the effects of host root (*Vicia faba* L.) extracts. He also crudely fractionated the host root exudate and showed the presence of both stimulatory and inhibitory material.

Several growth regulators and chemical substances have been observed to inhibit broomrape seed germination. Growth regulators, such as kinetin; coumarin and abscisic acid showed an inhibitory effect on *O. crenata* seed germination in the presence of the natural germination stimulants (30) and ethylene at high concentrations appeared to inhibit broomrape seed germination instead of stimulating it (20). A number of phenolic substances

belonging to the hydroxycinnamic acids that occur mostly in a non-free state and caffeic acid and its quinnic acid ester, chlorogenic acid, protocatechuic acid and a number of esters of glycosides of other derivatives were identified in water solutions that stimulated the germination of *O. crenata* after a period of soaking the seeds at 20° C for 15 to 30 days. An inverse relationship was found to exist between the phenolic content of the preconditioned seeds and their germination, suggesting that phenolic substances acted as seed germination inhibitors (31).

2.3.3.2 *Control of Broomrape After Attachment to Host Roots*

Several herbicides have been tested for the control of broomrape during its subterranean period of development on host roots. Among the soil applied herbicides, preplanting applications of chlorpropham, dalapon and TCA have been reported as effective against broomrape. Diphenamid and mixtures of diphenamid and trifluralin have been shown to reduce broomrape infestation in tobacco (54, 101, 102). However, all treatments were toxic to crop plants. Trifluralin incorporated before seedbed preparation reduced the number of broomrape shoots emerged by 70 to 80% (115). In another experiment, trifluralin or diphenamid injected around tobacco roots 35 days after transplanting proved effective in reducing broomrape parasitism. None of the treatments injured tobacco plants (91, 92, 93, 94). In some other experiments, however, trifluralin and some other herbicides such as dinitramine, pendimethalin, nitralin, oryzalin, pronamide, dichlobenil, and dinoseb in potato (61) or broad bean (58) proved ineffective. Oxadiazon applied through sprinkler irrigation to the root zone of tomato was somewhat effective in controlling broomrape (61).

Some reports have indicated that systemic herbicides such as maleic hydrazide and glyphosate can be used to control broomrape in some crops. These herbicides can be sprayed postemergence on crop plants so that they are translocated through the host plant to the parasite. Maleic hydrazide (triethanolamine salt) sprayed on tobacco leaves effectively

inhibited the development of *O. aegyptiaca* and *O. ramosa* parasitizing the crop roots and imparted immunity to host plants from further broomrape infestation for the rest of the season (35). Maleic hydrazide sprayed on watermelon foliage immediately after the attachment of *O. aegyptiaca* to host roots inhibited the growth of the parasite (90, 107). Parasitism of tobacco plants by broomrape increased incorporation of ^{14}C ($^{14}CO_2$) by host plants and accelerated the transport of photosynthates to leaves, roots and broomrape shoots. Maleic hydrazide reduced photosynthesis in tobacco leaves and the flow of ^{14}C assimilates to tobacco flowers and suckers and to broomrape plants. This resulted in an improved yield and quality of tobacco (35). Similar results were obtained with maleic hydrazide on tobacco by Sotnik and Okazawa (110). They sprayed maleic hydrazide (sodium salt) on tobacco when 30 to 40% of plants were in flower and supported *O. ramosa* plants at the bud or flowering stages on their roots. Over 80% of the *O. ramosa* plants were killed and the treated tobacco plants matured 7 to 10 days earlier than untreated plants.

2,4-D applied to the leaves of broad bean plants parasitized by *O. crenata*, translocated from the foliage to the roots and into the attached parasite (116). The developing tubercle of *O. crenata* was found to contain the herbicide in a concentration of fourteen times more than in the host roots, 5 days after treatment. The rate of 2,4-D applied to the bean plants, however, produced slight distortion of the stem apex and twisting of the leaf petioles but had no apparent effect on the tubercle development of broomrape. In laboratory experiments, higher concentrations of 2,4-D than those that were present in the developing tubercles were required to inhibit the elongation of broomrape radicles (116). Hence, due to the high susceptibility of most broadleaf crops to 2,4-D, the use of this herbicide for broomrape control is unlikely to prove useful. The fact, however, that the herbicide was translocated and accumulated in the parasite in concentrations much higher than in the host suggested that if a herbicide could be found that was no more toxic to the host than to the parasite, it could be used to control broomrape selectively in crops.

Glyphosate is a nonselective, postemergence herbicide that is readily translocated from the point of application on the leaves to all metabolically active parts of the plant (24, 39,

42). Due to its nonselective nature, glyphosate is generally applied in noncropped areas or prior to planting or after harvesting in croplands for broad spectrum weed control. This herbicide has been found to be very effective against broomrape. Very interestingly, when applied to crop plants such as broad bean at very low rates (60 to 120 g/ha), glyphosate has been observed to suppress and/or control the attached parasite without adversely affecting the host plants (105, 106).

The effectiveness of glyphosate as a foliar application for *O. crenata* control in broad bean was first reported by Kasasian (58). The rate of 200 g/ha provided complete control of the parasite and sufficient safety margin, indicating the relative tolerance of broad bean to the herbicide. Similar results were obtained with *O. aegyptiaca* control in tobacco (58). Several researchers have since confirmed that glyphosate could be used for selective control of broomrape in crops such as broad bean (49, 50, 105, 106).

The results of selective control of broomrape with glyphosate in some other crops such as pea, carrot, and tomato have, however, been less encouraging. Rates of the herbicide as low as 18 to 72 g/ha were observed to control *O. aegyptiaca* parasitizing tomato but the treatments injured the crop plants (49). Hence, the limiting factor to glyphosate deployment as a broomrape herbicide is the margin of selectivity in these crops.

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3.0 POTENTIAL OF THREE BROOMRAPES (Orobanche spp.) TO PARASITIZE FIVE MAJOR BROADLEAF CROPS

3.1 INTRODUCTION

Broomrape (*Orobanche* spp.) is a flowering plant that parasitizes the roots of many economically important plants (Figure 3.1). The genus belongs to the family Orobanchaceae. The members of this family are obligate parasites that lack chlorophyll and hence the ability to synthesize their own food (5). They draw nutrients and water from the host plants by attaching to their roots, consequently reducing the hosts' yield potential.

Many species of broomrape cause damage to agronomic and horticultural crops throughout the semi-arid and some temperate regions of the world. *O. aegyptiaca* Pers. (Egyptian broomrape), *O. ramosa* L. (branched broomrape), and *O. crenata* Forsk. (crenate broomrape) are three of the most devastating species of broomrape. The former two have a

Figure 3. 1. A mature plant of ***O. aegyptiaca*** parasitizing a tomato plant.

wide host range that includes members of the Fabaceae, Solanaceae, and Asteraceae families. *O. crenata* has a relatively narrow host range affecting most commonly members of the Fabaceae and Umbeliferae families. None of these broomrape species is known to attack monocotyledonous plants (5).

Presently, three broomrape species, *O. ramosa, O. minor* Smith, and *O. ludoviciana* Nutt., occur in the United States. *O. ramosa* and *O. minor* were introduced from the Old World, whereas *O. ludoviciana* is considered to be native to North America (5). *O. ramosa* was found in 1890 in Kentucky and later in New Jersey and in a greenhouse on Long Island, New York. Infestations of this parasitic weed reached devastating levels on tomato (*Lycopersicon esculentum* Mill.) crops in the Sacramento Valley of California in 1959, triggering an intensive control program involving fumigation of the soil with methyl bromide. It is still a recurring problem in that state. Recently, a highly virulent strain of *O. ramosa* was discovered in Karnes County, Texas (6). An intensive control program is underway to eradicate this infestation. *O. minor* is considerably less damaging than *O. ramosa*. Although it has been discovered in 10 eastern and two western states and the District of Columbia (3), it is not considered a major problem on any crop in the USA at present. However, a similar situation existed in New Zealand for many years, after which *O. minor* became established in clover (*Trifolium* spp.) and caused economic damage (4). *O. ludoviciana* is a parasite of commercial crops, such as tobacco (*Nicotiana tabacum* L.) and tomato, in Ohio, Wyoming, and California.

The objective of this investigation was to determine the potential of the world's three most devastating species of broomrape, i.e., *O. aegyptiaca*, *O. ramosa*, and *O. crenata* to parasitize five major broadleaf crops of Virginia in greenhouse studies.

3.2 MATERIALS AND METHODS

3.2.1 Influence of Potting Media on Parasitism of Tomato Plants by Broomrape

Broomrape requires specific conditions for optimum infestation of host plants. In order to test which potting medium was best suited to study broomrape parasitism, four types of potting media were tested in the greenhouse. These potting media were (A) clay loam (33.3%), sand (33.3%) and Weblite (expanded shale; 33.3%); (B) clay loam (45%), sand (45%) and peat moss (10%); (C) vermiculite (40%), Weblite (40%) and peat moss (20%); and (D) sand alone. No fertilizer was added to potting media A and B, whereas, Osmocote (14-14-14) and (4-9-3) at 2.6 kg/m^3 and lime at 1.3 kg/m^3 were mixed with potting medium C. Potting medium D received 1/4 strength Hoagland's nutrient solution twice a week during the period of the experiment.

Tomato (var. Rutgers) plants at the two-true-leaf stage were transplanted in plastic pots containing the potting media. In order to obtain adequate infection of tomato plants with broomrape, 5 mg of *O. aegyptiaca* seeds were sprinkled directly on the roots of crop plants at the time of transplanting. *O. aegyptiaca* seeds used in these experiments were obtained from infested tomato plants in the Massua region of Israel in 1974. The pots were watered as needed to maintain normal plant growth. The temperature in the greenhouse ranged from 22 to 30° C and the day length was approximately 16 h. There were twelve replications of each potting medium and each pot contained two tomato plants. The experiment was repeated once with potting media A, B, and C.

Observations on the emergence of broomrape plants above the soil surface were recorded. When most of the broomrape plants had emerged, tomato plants were carefully

removed from the pots and the soil was gently washed off from the roots and the attached broomrape plants. Observations recorded on broomrape were the number of infections per plant and the mean length and fresh weight of broomrape shoots. Tomato plants were measured for shoot height and shoot and root fresh weight. The number of plants for each observation ranged from four to twelve. The data were analyzed statistically and the means were compared using the least significant difference at the 5% level of significance.

3.2.2 Parasitism of Various Crops with Three Species of Broomrape

Two to three week old seedlings of tomato (var. Rutgers), tobacco (var. Burley 21), alfalfa (*Medicago sativa* L. var Cimmaron), peanut (*Arachis hypogaea* L. var. Florigiant), and soybean (*Glycine max* L. var. Essex) were transplanted into 15-cm diameter plastic pots containing the potting medium A described in the above experiment. It consisted of equal volumes of clay loam, sand, and expanded shale (Weblite). Each pot contained either one or two plants.

O. aegyptiaca seeds were collected from the Massua region in 1974 and *O. ramosa* seeds were collected from the En-Ziza region in Israel in 1976. *O. crenata* seeds were obtained from Syria in 1982. Broomrape seeding was done at the time of transplanting the crop plants by placing about 5 mg of broomrape seeds in the root zone of the crop plants such that some broomrape seeds contacted the plant roots directly. The experiment consisted of six replications of each broomrape species on each crop. The temperature in the greenhouse ranged from 22 to 30°C and the day length was approximately 16 h. The pots were watered as needed to sustain normal plant growth. The experiment was performed twice.

A separate experiment in which infected and noninfected plants were compared was conducted to determine the effect of broomrape parasitism on tomato plants. Tomato plants (var. Rutgers) with two-true-leaves were transplanted in 15-cm diameter plastic pots as above.

One set of plants was not inoculated with broomrape seeds while the other set was inoculated with *O. aegyptiaca* seeds.

Observations on the number of broomrape infections per plant, shoot height and fresh weight of broomrape and crop plants, and root fresh weights of crop plants were recorded 10 weeks after inoculation. The means were compared using the Duncan's Multiple Range test and orthogonal contrasts at the 5% level of significance. The experiments were conducted under strict quarantine conditions approved by the USDA, APHIS-PPQ, USA. At the end of the experiments, all plant material, soil, and equipment were sterilized to devitalize broomrape seeds before discarding.

3.3 RESULTS AND DISCUSSION

3.3.1 Influence of Potting Media on Broomrape Parasitism

The growth of tomato plants in the four types of potting media differed significantly. Tomato plants in potting medium C grew much more vigorously than tomato plants in potting medium A, B or D as evidenced by their shoot and root fresh weights (Table 3.1). In general, broomrape parasitism on tomato plants occurred much more and sooner in potting media A and B than in potting medium C or D. Broomrape shoots were visible in about 6 weeks in almost all pots containing potting medium A or B. Broomrape shoots emerged in about 7 weeks in only two of the 12 pots containing potting medium C and no emergence of broomrape shoots was noticed in potting medium D. There were three or more broomrape infections on tomato plants in potting media A and B (Table 3.2). The shoot height and fresh weights of broomrape plants did not differ significantly in potting media A and B.

Table 3. 1. Effect of four potting media on growth of 'Rutgers' tomato plants.

Treatment†	Shoot‡ height	Shoot‡ F.W.	Root‡ F.W.
	(cm)	(g)	(g)
Potting medium A	11.98	3.40	1.36
Potting medium B	12.65	2.65	0.96
Potting medium C	198.75	210.97	9.92
Potting medium D	18.98	2.52	1.52
LSD (0.05)	43.55	48.25	2.17

† Potting medium A = Clay loam (33.3%), Sand (33.3%), Weblite (33.3%)
Potting medium B = Clay loam (45%), Sand (45%), Peat moss (10%)
Potting medium C = Vermiculite (40%), Weblite (40%), Peat moss (20%)
Potting medium D = Sand
Potting medium C contained fertilizers (14-14-14) and (4-9-3) at the rate of 2.6 kg/m^3 and lime at 1.3 kg/m^3. Potting medium D was supplied with 1/4 strength Hoagland's nutrient solution. Potting media A and B did not contain any fertilizer.

‡ Numbers are means of six replications.

Table 3. 2. Effect of four potting media on the growth of ***O. aegyptiaca*** plants parasitizing on tomato var 'Rutgers' plants.

Treatment†	Infections‡ per plant	Shoot‡* height	Shoot‡ F.W.
	(cm)	(g)	
Potting medium A	3.42	10.67	2.57
Potting medium B	3.00	10.57	2.22
Potting medium C	0.08	0.50	0.32
Potting medium D	0.90	0.00	0.05
LSD (0.05)	0.90	3.00	0.70

† Potting medium A = Clay loam (33.3%), Sand (33.3%), Weblite (33.3%)
Potting medium B = Clay loam (45%), Sand (45%), Peat moss (10%)
Potting medium C = Vermiculite (40%), Weblite (40%), Peat moss (20%)
Potting medium D = Sand
Potting medium C contained fertilizers (14-14-14) and (4-9-3) at the rate of 2.6 kg/m^3 and lime at 1.3 kg/m^3. Potting medium D was supplied with 1/4 strength Hoagland's nutrient solution. Potting media A and B did not contain any fertilizer.

‡ Numbers are means of six replications.

* Shoot height is height of broomrape shoots above the potting medium surface.

The more vigorous growth of tomato plants in potting medium C may be the reason for their tolerance to broomrape infection. It has been reported that plants growing in fertile soils are more tolerant to broomrape infection than plants growing on soils with marginal fertility (1). Potting medium C was adequately fertilized with nitrogen, phosphorus, and potassium; whereas, potting media A and B did not receive any additional fertilizer. Also, the physical characteristics of the media, such as the water holding capacity, may have influenced broomrape infection on tomato plants. Potting medium C with a higher vermiculite and peat moss content had a greater water holding capacity than potting medium A or B.

3.3.2 Parasitism of Five Crops with Three Species of Broomrape

The effect of broomrape parasitism on the growth of host plants was determined by contrasting infected and noninfected plants only in tomato due to its known susceptibility to the parasite. Broomrape-infected tomato plants showed significantly lower shoot height, shoot fresh weight (F.W.) and root length than that of noninfected tomato plants (Table 3.3). Although root fresh weight of broomrape-infected tomato plants was lower than that of noninfected tomato plants, this difference was not statistically significant. Of the three species of broomrape tested, only *O. aegyptiaca* (Figure 3.1) and *O. ramosa* parasitized tomato plants (Table 3.4). *O. aegyptiaca* shoots emerged above the soil surface were considerably taller than *O. ramosa* shoots; however, *O. ramosa* shoots weighed almost twice as much as *O. aegyptiaca* shoots. There were no significant differences in the effects of the two broomrape species on the growth of tomato plants.

Tobacco plants were parasitized by all three broomrape species. However, they were considerably more susceptible to *O. aegyptiaca* (Figure 3.2) and *O. ramosa* than to *O. crenata*. Only one *O. crenata* plant was found parasitizing the roots of a tobacco plant in this experiment. *O. ramosa* parasitized tobacco plants in greater numbers than did *O.*

Table 3. 3. Effect of ***O. aegyptiaca*** parasitism on growth of 'Rutgers' tomato plants.

Treatment	Tomato†				Broomrape†		
	Shoot height	Shoot F.W.	Root length	Root F.W.	Infections per plant	Shoot‡ height	Shoot F.W.
	(cm)	(g)	(cm)	(g)		(cm)	(g)
No-Inoculation	26.2	3.1	21.6	0.84			
Broomrape-Infection	13.8	1.2	6.6	0.49	2.58	5.2	0.56
LSD (0.05)	4.4	0.7	4.9	0.37			

† Numbers are means of six replications.

‡ Broomrape shoot height above the surface of the potting medium.

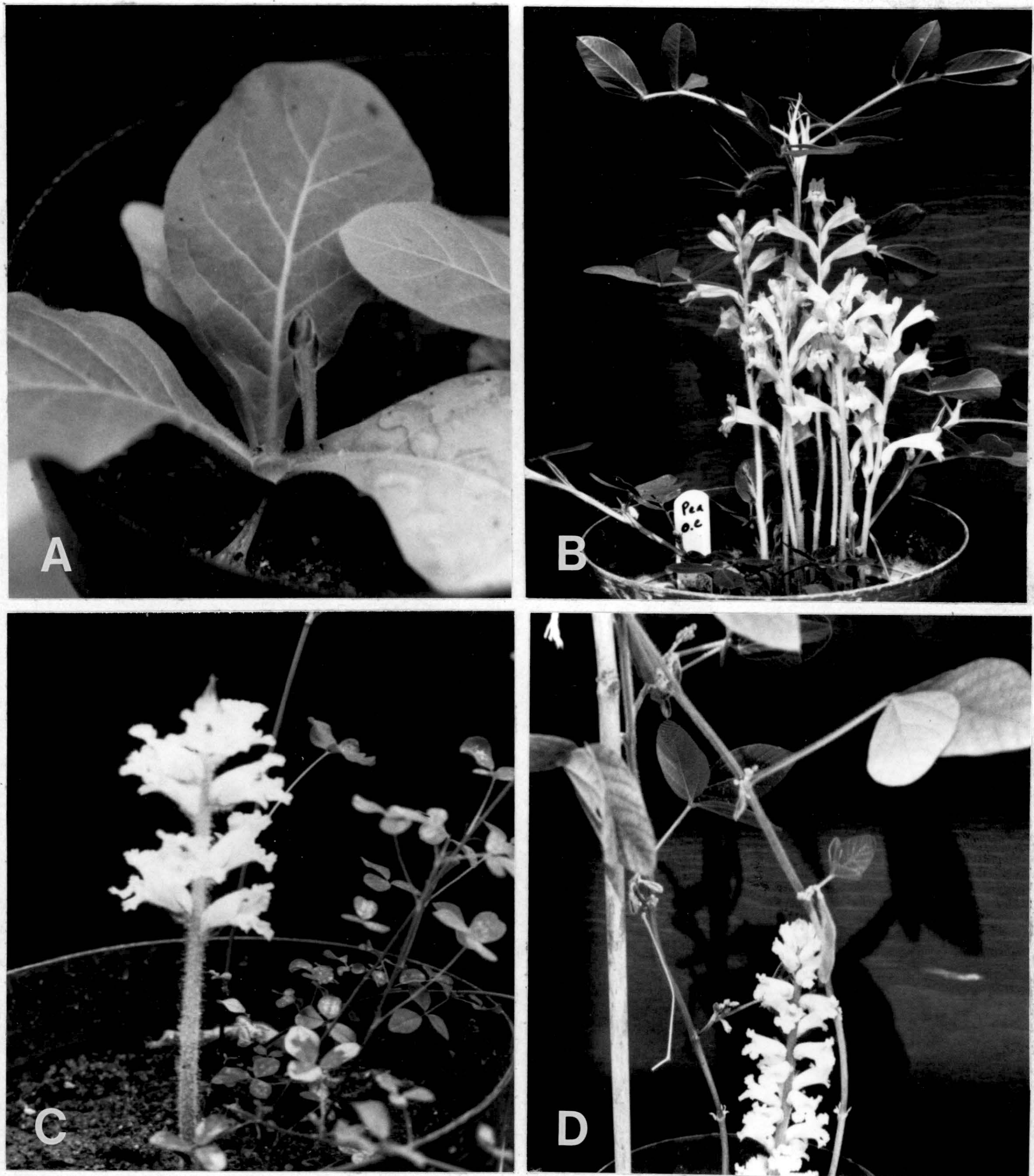

Figure 3. 2. (A) ***O. aegyptiaca*** parasitizing a tobacco plant, (B) ***O. aegyptiaca*** parasitizing a peanut plant, (C) ***O. crenata*** parasitizing an alfalfa plant, and (D) ***O. crenata*** parasitizing a soybean plant.

Table 3. 4. Parasitism of five crops with three species of broomrape.

Broomrape Species	Crop†			Broomrape†		
	Shoot height	Shoot F.W.	Root F.W.	Infections per plant	Shoot‡ height	Shoot F.W.
	(cm)	(g)	(g)		(cm)	(g)
			Tomato			
O. aegyptiaca	13.42 b	0.98 b	0.51 a	2.0 a	5.54 a	0.62 b
O. ramosa	16.25 b	1.14 b	0.63 a	2.4 a	1.29 a	1.13 b
O. crenata	22.75 a	2.29 a	0.67 a	0.0 b	0.00 b	0.00 a
			Tobacco			
O. aegyptiaca	8.63 a	1.88 a	1.26 a	1.3 a	1.70 a	0.58 a
O. ramosa	7.59 a	1.92 a	0.94 ab	2.0 a	0.18 a	0.33 a
O. crenata	8.04 a	2.11 a	0.59 a	0.1 b	0.00 b	0.01 b
			Alfalfa			
O. aegyptiaca	36.67 a	2.38 a	1.40 a	0.18 a	2.67 a	0.38 a
O. ramosa	38.50 a	3.33 a	1.93 a	0.00 b	0.00 b	0.00 b
O. crenata	42.83 a	3.42 a	2.58 a	0.08 a	0.92 a	0.13 a
			Peanut			
O. aegyptiaca	25.17 a	14.56 a	4.40 a	0.18 a	2.67 a	0.38 a
O. ramosa	25.17 a	15.65 a	3.30 a	1.50 a	0.00 b	0.82 a
O. crenata	22.17 a	13.60 a	3.22 a	1.83 a	4.77 ab	6.06 a
			Soybean			
O. aegyptiaca	114.1 a	16.40 a	9.87 a	0.00 b	0.00 b	0.00 b
O. ramosa	86.9 b	15.70 a	8.03 a	0.08 a	0.00 b	0.05 a
O. crenata	86.8 b	14.60 a	7.73 a	0.08 a	1.25 a	0.60 a

† Means within a column and for each host followed by the same letter are not significantly different according to Duncan's Multiple Range Test at $P \leq 0.05$. Data are averages of six replications.

‡ Shoot height of broomrape refers to height above the soil surface. In the case of soybean, only one broomrape shoot emerged which reached a height of 15 cm.

aegyptiaca, although this difference was not statistically significant (Table 3.4). There were no apparent differences in the effect of either species on the growth of tobacco plants as compared to the plants scarcely infected by *O. crenata*.

Alfalfa was parasitized by *O. aegyptiaca* and *O. crenata* (Figure 3.2), but not by *O. ramosa*. Alfalfa was a poor host but appeared to be more susceptible to *O. aegyptiaca* than to *O. crenata* as evidenced by the number of infections and shoot weight of broomrape, although these differences were not statistically significant (Table 3.4). The shoot and root growth of alfalfa plants infected by broomrape were not significantly different from the shoot and root growth of alfalfa plants inoculated with but not infected by *O. ramosa*.

Peanut appeared to be most susceptible to broomrape among all five crops tested; it was parasitized by all three species of broomrape (Figure 3.2 and Table 3.4). *O. aegyptiaca* and *O. crenata* appeared more vigorous than *O. ramosa* on peanut. However, there was no difference in the growth and pod yield (data not shown) of peanut plants parasitized by the three broomrape species.

In contrast to peanut, soybean plants appeared to be somewhat resistant to broomrape infection (Table 3.4). Only one plant each of *O. ramosa* and *O. crenata* was found parasitizing soybean plants. Once attached, however, *O. crenata* grew vigorously on soybean up to a height of about 15-cm above the soil surface (Figure 3.2). *O. ramosa*, however, formed a small tubercle on soybean roots and had not emerged above the soil surface by the time of harvesting, 10 weeks after inoculation. The height of soybean plants infected with *O. crenata* or *O. ramosa* was significantly lower than that of plants inoculated but not infected with *O. aegyptiaca*. No infection from any of the three species was found on soybean plants in the repeat experiment.

In general, shoots of *O. aegyptiaca* and *O. ramosa* emerged earlier than those of *O. crenata*. The former species emerged within 6 weeks, while *O. crenata* emerged within 8 weeks of inoculation on crop roots. Also *O. aegyptiaca* and *O. ramosa* showed a greater host range than *O. crenata* by infecting plants belonging to both Solanaceae and Fabaceae families. *O. crenata* was mainly restricted to members of the Fabaceae family, although one

O. crenata infection was noticed on tobacco roots. It has been reported that *O. aegyptiaca* parasitizes a wide range of host plants but *O. ramosa* infects plants belonging mainly to the Solanaeceae family (5). Few reports exist in the literature of broomrape parasitism on peanut or soybean. This may be because these crops are not commonly grown in the worst broomrape-infested areas of the world such as the Middle East and Southern Europe. *O. crenata* is known to parasitize broad bean (*Vicia faba* L.), and pea (*Pisum sativum* L.) in Southern Europe and the Mediterranean region (5). *O. aegyptiaca* is known to parasitize peanut in Israel (Jacobsohn, personal communication).

The potting medium used in this experiment contained no added fertilizer. It is known that broomrape infection is most severe on low fertility soils (1, 2). and that leguminous crops growing on infertile soils develop nodules more readily than plants growing on fertilized soils. *Rhizobium* infection and broomrape (*O. crenata*) penetration often coincide, and nodules constitute a route by which broomrape could by-pass the root rhizodermis and gain infection (7). Soybean plants in our experiments developed numerous nodules. It is thus possible that nodulation on soybean roots may have enhanced the ability of broomrape to infect the plants. Poor fertility of the potting medium was also responsible for slow growth of Solanaceous crop plants, which may have masked the effect of broomrape infection on shoot and root growth.

High inoculum density of broomrape seed may also enhance parasitism of soybean plants. The amount of broomrape seed used to inoculate crop plants in my experiments was fairly high as compared to what would be encountered naturally by crop plants in the soil. It has been reported that high inoculum density, such as that of *Cylindrocladium crotalariae* microsclerotia and the soybean cyst nematode (*Heterodera glycines*), can result in high disease incidence or a few infections, respectively, on the roots of plants that under other circumstances may be nonhosts (8, 9). Williams (10), however, reported that high inoculum levels of witchweed (*Striga senegalensis* Benth.) did not increase the number of infections on sorghum (*Sorghum vulgare* L.), which is highly susceptible to witchweed, but did cause a greater loss to the host than did low inoculum levels. The effects of density of broomrape

seeds on germination, attachment, parasitism of the host plant are still not well understood and require further study.

These results confirm that, under ideal conditions for broomrape growth, the world's three most devastating species of broomrape, if allowed to be introduced into new areas, have the potential to parasitize several major broadleaf crops and could cause significant damage to the country's agriculture.

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4.0 SOME FACTORS AFFECTING BROOMRAPE SEED GERMINATION

4.1 INTRODUCTION

Broomrapes lack chlorophyll and are well adapted to the obligate mode of parasitism, which involves survival of broomrape seeds in the soil for long periods of time, host recognition, and early attachment and penetration into a source of nutrition. The seeds released from the mother plant are dormant and do not germinate until a suitable host or a non-host "trap" plant is present in their vicinity (3).

The germination process of broomrapes is quite complex and involves at least three phases. The first phase is commonly called 'after-ripening' or 'post-harvest ripening'. Little precise data on after-ripening are available (12). This ripening is the period of time between shedding of seeds and the second stage of conditioning, also referred to as preconditioning or pretreatment. During the preconditioning phase, the seeds become receptive to a germination stimulant after being incubated under suitable temperature and moisture for a

few days to 2 weeks. The third phase is the stimulant treatment phase during which the seeds germinate by producing a germ-tube-like radicle.

Reports in the literature on broomrape seed germination are inconsistent. Germination percentages of the various species of broomrape have ranged from a low 40% to as high as 90% (8, 10, 13, 17, 18). The factors that affect the various processes of broomrape seed germination are not fully understood. Kasasian (10) reported that good germination of *O. aegyptiaca* Pers. and *O. crenata* Forsk. was obtained over quite a wide range of temperatures. However, the optimum temperature for germination of *O. aegyptiaca* seeds was 23°C. The length of the preconditioning period also affects germination of broomrape seeds. *O. aegyptiaca* seeds germinated well as long as the preconditioning period was 9 days or longer. *O. crenata* seeds required at least 11 days of preconditioning before maximum germination was obtained in response to a germination stimulant (10). Exudates of sorghum (*Sorghum vulgare* L.) and broad bean (*Vicia faba* L.) gave optimum germination of *O. aegyptiaca* between pH 4 and 5 (10).

The objectives of this investigation were to test various methods and to determine optimum conditions for germination of broomrape seeds in the laboratory.

4.2 MATERIALS AND METHODS

4.2.1 Source of Broomrape Seeds

O. aegyptiaca seeds used in these experiments were obtained from Israel under license from APHIS-PPQ, USA. Two different seed lots of *O. aegyptiaca* were used in these investigations. One lot contained seeds collected from broomrape-parasitized tomato plants

in the Massua region of Israel in 1974. The other lot contained seeds collected from broomrape parasitizing potato plants in the Shave Zion region of Israel in 1978. *O. ramosa* L. seeds were collected from En-Ziza region in Israel in 1976 and *O. crenata* seeds were obtained from Syria in 1982.

4.2.2 Surface Sterilization of Broomrape Seeds

Four different methods of surface sterilization of broomrape (*O. aegyptiaca*) seeds were tested. These methods consisted of treatment of dry broomrape seeds with sodium hypochlorite (2.5%) alone or Sporicidin (glutaraldehyde-phenate solution diluted 1:16 to a glutaraldehyde level of 13%. An activator solution was added to the diluted glutaraldehyde-phenate solution each time just prior to use. The product was obtained from The Sporicidin Company, 4000 Massachusetts Ave, NW, Washington, DC 20016) alone, or treatment with sodium hypochlorite (2.5%) or Sporicidin combined with mild sonication. The seeds were surface sterilized with sodium hypochlorite or Sporicidin by placing them in the sterilizing solutions for 10 minutes. In the other two treatments, seeds placed in sodium hypochlorite or Sporicidin solution were subjected to mild sonication for 1 minute and then left in the sterilizing solution for 10 minutes. The solutions along with the seeds were drained on a filter paper placed in a Buchner funnel attached to a filtration flask. The sterilizing solutions were filtered out under vacuum and the seeds were washed with several flushes of sterile distilled water. The filter paper containing the seeds was removed from the Buchner funnel and placed in a clean petri dish. The seeds were allowed to dry before they were used for germination tests.

4.2.3 Methods of Germinating Broomrape Seeds

Three different methods were tested for studying broomrape (*O. aegyptiaca*) seed germination in the laboratory. The first method involved plastic culture plates with 24 wells in which surface sterilized broomrape seeds were immersed in sterilized distilled water for 2 weeks to allow for preconditioning. After the preconditioning period, distilled water in the wells was replaced by strigol analog solutions and incubated in dark for 1 week before germination was counted. The preparation of strigol analog solutions is described later in this section. The seeds were incubated at 22 to 25°C during preconditioning and strigol analog treatment.

The second method involved germinating broomrape seeds on 2% agar medium in glass petri dishes. Broomrape seeds were sprinkled directly on the agar medium and allowed to incubate at 22 to 25°C. Strigol analogs at various concentrations were incorporated with the agar at the time of preparation of the medium. Percent germination was obtained after 3 weeks of incubation.

The third method of germinating broomrape seeds consisted of 1-cm glass fiber filter paper (GFFP) disks, placed on a single sheet of Whatman 5 filter paper in a 9-cm diameter glass petri dish. Each GFFP contained about 25 broomrape seeds and there were 25 GFFP disks in each petri dish. Each petri dish was supplied with 3 ml of distilled water. The petri dishes were placed in a plastic container wrapped in aluminum foil to exclude all light. The seeds were incubated at 22 to 25°C for 2 weeks. After the preconditioning period, the GFFP disks containing the broomrape seeds were removed from the petri dishes and placed on new GFFP disks each soaked with 50 µl solution of the growth regulator or a control solution. The control solution contained 0.1% dimethyl sulfoxide (DMSO). About 10 GFFP disks with the seeds were placed in each petri dish. All treatments were carried out under 'safe' green light, and the treated seeds were incubated in the dark at 22 to 25°C. Germination percentage was obtained 1 week after treatment with the strigol analogs.

Strigol analog solutions were prepared by first dissolving the compounds in a small volume (approx. 1 ml) of pure dimethyl sulfoxide (DMSO) and then adding sufficient distilled water to obtain the desired strigol analog concentrations. The final DMSO concentration in strigol analog solutions was adjusted to 0.1% by volume and the pH of the solutions was adjusted to 6.5 using dilute (0.01 M) HCl and NaOH.

4.2.4 Effect of Strigol Analogs, Light, and pH on Broomrape Seed Germination

The effect of various factors on germination of broomrape seeds was investigated by using the GFFP disk method. As described above, broomrape seeds, surface sterilized with Sporicidin were preconditioned on GFFP disks soaked with distilled water. The petri dishes containing the seeds were placed in a plastic container which was wrapped in aluminum foil to exclude all light. To study the effect of light on seed germination, the plastic container with a set of treatments was left uncovered and placed under continuous light in an incubator. The seeds were incubated at 22 to 25°C for ten days.

After the preconditioning period, broomrape seeds were treated with the strigol analogs, GR 7 or GR 24. The concentrations of the strigol analogs ranged from 0.01 to 10.0 ppm (equivalent to 0.05 - 50 μM). Treatment of broomrape seeds with the growth regulators was carried out under 'safe' green light and the treated seeds were incubated in the dark at 22 to 25°C.

Two experiments were done to study the effect of pH, on germination of *O. aegyptiaca* seeds. In the first experiment, the pH of the preconditioning solution prepared in KH_2PO_4/NaOH buffers ranged from 4.5 to 8.0. In the experiment conducted to determine the time period required after treatment with the strigol analogs for maximum germination, germination was counted 1, 2, 3, 5, 7, and 14 days after treatment with the strigol analogs. In

all other experiments, germination percentage was obtained 1 week after treatment with the strigol analogs. The results were analyzed using the F test, and the differences in means were separated using the least significant difference (LSD) test at the 5% level. All experiments were repeated at least once.

4.3 RESULTS AND DISCUSSION

4.3.1 Surface Sterilization of Broomrape Seeds

O. aegyptiaca seeds showed a significant spontaneous germination (germination that occurs after surface sterilization and preconditioning, but without the addition of a known germination stimulant). Seeds surface sterilized with sodium hypochlorite showed about 5% spontaneous germination (Table 4.1). Seeds surface sterilized with sodium hypochlorite and subjected to mild sonication showed three times higher spontaneous germination than seeds surface sterilized with sodium hypochlorite but not subjected to sonication. Seeds surface sterilized with Sporicidin showed as high spontaneous germination with or without sonication as seeds surface sterilized with sodium hypochlorite and sonication.

Surface sterilization of seeds with sodium hypochlorite resulted in a maximum germination of about 35% following treatment with GR 24 at 0.1 ppm. Surface sterilization of seeds with sodium hypochlorite and sonication, in general, resulted in a slightly higher germination than that of seeds surface sterilized with sodium hypochlorite and not subjected to sonication. However, these differences in germination were not statistically significant, except when the seeds were treated with GR 24 at 0.001 ppm.

Table 4. 1. Effect of GR 7 and GR 24 using four different surface sterilization methods on ***O. aegyptiaca*** seed germination.

Treatment	Sodium hypochlorite	Sodium hypochlorite + sonication	Sporicidin	Sporicidin + sonication	LSD (0.05)
	--------------------	% germination†	--------------------		
Control	5.3	15.2	14.2	13.9	7.0
GR 7 0.10 ppm	17.4	29.7	53.2	34.4	12.8
GR 7 1.00 ppm	17.5	29.7	57.0	36.8	14.3
GR 7 10.0 ppm	29.4	37.7	55.7	43.3	17.6
GR 24 0.01 ppm	23.6	38.9	50.8	43.3	11.4
GR 24 0.10 ppm	35.3	38.9	54.0	46.2	19.9
GR 24 1.00 ppm	29.8	29.5	53.5	40.6	18.0
LSD (0.05)	5.9	5.3	9.2	11.3	

† Numbers are means of four replicates. Each replication consisted of about 100 seeds.

Surface sterilization of seeds with Sporicidin, in general, gave higher percent germination of *O. aegyptiaca* seeds than surface sterilization with sodium hypochlorite. The maximum germination of seeds with Sporicidin was about 57% following treatment with GR 7 at 1.0 ppm or GR 24 at 0.1 ppm. Unlike in the case of sodium hypochlorite, however, seeds surface sterilized with Sporicidin and sonication showed slightly lower percent germination following treatment with strigol analogs than seeds surface sterilized with Sporicidin but not subjected to sonication. There was no apparent effect of sonication on spontaneous germination of *O. aegyptiaca* seeds surface sterilized with Sporicidin.

Surface sterilization of broomrape seeds is necessary to protect the seeds from microbial contaminants that often obscure results, as well as create an unfavorable environment by altering pH, digesting the seed, or producing toxic substances. Both sodium hypochlorite and Sporicidin were effective in surface sterilizing broomrape seeds. Sodium hypochlorite combined with mild sonication, however, was more effective in surface sterilizing broomrape seeds than sodium hypochlorite alone. Surface sterilization with sodium hypochlorite and sonication also resulted in a higher spontaneous germination than surface sterilization with sodium hypochlorite alone. Sodium hypochlorite has been shown to break dormancy of many weed seeds including those of *Aeginatia indica* L. (Orobachaceae) (6) and *Striga asiatica* (9); and calcium hypochlorite causes germination of broomrape seeds (4). The spontaneous germination of *O. aegyptiaca* seeds observed in these experiments may have been partly due to treatment with sodium hypochlorite or Sporicidin.

Sonication of broomrape seeds immersed in sodium hypochlorite may have made the seed coat more permeable which probably helped penetration of water and strigol analogs into the seed. This may have been the reason for higher percent germination when the seeds were subjected to sonication in sodium hypochlorite solution. There was no such effect of sonication on germination when the seeds were surface sterilized with Sporicidin. The reason for higher percent germination obtained with Sporacidin as compared to sodium hypochlorite is not known. Sodium hypochlorite treatment also resulted in bleaching of the seed coat which made counting of germinating seeds on GFFP disks difficult. Surface sterilization with

Sporicidin did not result in the bleaching of the seed coat. It was used as a surface sterilizing agent in these experiments at the recommendation of Prof. James Riopel (Department of Biology, University of Virginia). An effective sterilizing action combined with higher germination and no bleaching action on the seed coats made Sporicidin an ideal choice for surface sterilization of broomrape seeds.

4.3.2 Methods of Broomrape Seed Germination

Of the three methods for broomrape seed germination, the GFFP disk method gave significantly higher percent germination of *O. aegyptiaca* seeds than the culture plate or the agar medium method (Table 4.2). No spontaneous germination occured in the culture plates and a very low percent of broomrape seeds (a maximum germination 3%) germinated following treatments with the strigol analogs. Therefore this method did not prove very useful in studying germination of broomrape seeds.

The culture plate method has been used for studying germination of witchweed (*Striga* spp.) seeds and has proved useful in examining the haustorium initiation and early development in *Agalinis purpurea* (L.) RAF (Scrophulariaceae) and witchweed (2). Low germination of *O. aegyptiaca* seeds in the culture plates may be due to the reason that the seeds were kept completely immersed during preconditioning and during stimulant treatment. Broomrape, particularly *O. aegyptiaca*, being adapted to the semi-arid climate probably does not respond well to excess moisture.

Germination of *O. aegyptiaca* seeds in the agar medium was significantly higher than in the culture plate method. Although only a few seeds showed germination spontaneously, germination of strigol analog treated seeds was as high as 36% following treatment with GR 24 at 10 ppm (Table 4.2). Agar medium has been used to study germination of broomrape seeds by several workers. Nash and Wilhelm (13) obtained a low (about 17%) germination of *O. ramosa* seeds when incubated on an agar medium containing 100 ppm gibberellic acid.

Table 4. 2. Germination of ***O. aegyptiaca*** seeds with GR 7 and GR 24 using three different methods.

Treatment	Culture plates	Agar medium	GFFP disks	LSD (0.05)
	------------ % germination† ------------			
Control	0.0	0.1	15.8	5.8
GR 7 1.00 ppm	1.9	20.4	52.9	17.9
GR 7 10.0 ppm	3.0	24.2	37.5	10.2
GR 24 1.00 ppm	1.5	28.6	46.6	12.1
GR 24 10.0 ppm	2.6	36.5	54.6	11.5
LSD (0.05)	1.2	10.1	19.8	

† Numbers are means of four replicates. Each replication consisted of at least 100 seeds.

Abu-Shakra et al. (1) observed upto 81% germination of *O. ramosa* seeds following soaking the seeds in a solution containing 100 ppm gibberellic acid and then incubation on an agar medium containing flax root exudates. One disadvantage of germinating seeds on the agar medium is that the germination stimulants, such as GR 7 and GR 24 used in my experiments are incorporated in the agar medium at the beginning of the preconditioning period. Pre-treatment of broomrape seeds with a germination stimulant, such as flax root exudate, is less effective than preconditioning in water followed by treatment with a germination stimulant in inducing germination of broomrape seeds (1). It is generally thought that broomrape seeds must undergo certain metabolic changes during the preconditioning period before they can respond fully to an external source of a germination stimulant. The GFFP disk method gave the highest percent germination of all the three methods tested. About 16% broomrape seeds germinated spontaneously on the GFFP disks. This method allowed one third more seeds to germinate following treatment with a strigol analog than the agar medium method. This method was first used by Kasasian and Parker (11) to test the effect of numerous herbicides on the germination of *O. aegyptiaca* and *Striga hermontheca* seeds. Since then it has been used in several investigations on broomrape seed germination with good results (7, 10, 17). The GFFP disk method provides the flexibility of preconditioning broomrape seeds in water or any treatment solution followed by treatment with a germination stimulant. This method was used for studying broomrape seed germination in all later experiments in these investigations.

4.3.3 Effect of Strigol Analogs, Light, and pH on Broomrape Seed Germination

The effect of GR 7 and GR 24 was investigated on the germination of *O. aegyptiaca*, *O. ramosa*, and *O. crenata* seeds. The former two species, *O. aegyptiaca* and *O. ramosa*,

showed some spontaneous germination. *O. crenata* seeds, however, did not show any spontaneous germination (Table 4.3). All three species responded to germination stimulation by both GR 7 and GR 24. *O. ramosa* showed maximum germination following treatment with 1 ppm GR 7 or 0.01 ppm GR 24. *O. aegyptiaca* responded best to 1 ppm GR 7 or 0.1 ppm GR 24. *O. crenata* appeared to be least sensitive to the strigol analogs among the three species. Maximum germination of *O. crenata* seeds was obtained with 10 ppm GR 7 or 1 ppm GR 24.

All three species of broomrape showed a relatively low percent germination. This may be due to the ageing of broomrape seeds. From these results, it is apparent, however, that *O. ramosa* is most sensitive while *O. crenata* is least sensitive to germination stimulation by the strigol analogs. No report is available in the literature that compares the sensitivity of various broomrape species to GR 7 and GR 24. In general, GR 24 is slightly more effective than GR 7 in stimulating germination of broomrape seeds. This is perhaps due to the close similarity between the structure of GR 24 and that of strigol, the natural germination stimulant. The structure of GR 7 does not resemble the structure of strigol as closely as that of GR 24 (see Figure 2.3).

Due to the low germination of broomrape seeds obtained in the above experiment, another seed lot (*O. aegyptiaca* collected in 1978) was used to determine the time required for maximum germination after exposure to the strigol analogs. The seeds were preconditioned in water for ten days and then treated with GR 7 or GR 24. Germination was counted 1, 2, 3, 5, 7, and 14 days after treatment with the strigol analogs (Table 4.4). When no strigol analogs were applied, a maximum spontaneous germination of 9.6% was observed 14 days after post conditioning treatment. Treatment with strigol analogs resulted in a maximum germination of about 80%. *O. aegyptiaca* seeds began to germinate within one day of treatment with the strigol analogs. However they reached maximum germination within 3 days after treatment with GR 24 at 0.001 ppm and within 7 days after treatment with GR 7 at 0.01 ppm. In general, GR 24 appears to be slightly faster acting than GR 7. Both strigol analogs were effective, however, in bringing about maximum germination of broomrape seeds at the lowest concentration used, indicating that only minute quantities of the strigol analogs were required

Table 4. 3. Effect of GR 7 and GR 24 on the germination of three species of broomrape.

Treatment	*O. aegyptiaca*†	*O. ramosa*†	*O. crenata*†	LSD (0.05)
	------------ % germination ------------			
Control	8.0	9.1	0.0	6.5
GR 7 0.1 ppm	12.7	22.3	1.4	10.3
GR 7 1.0 ppm	21.3	29.6	7.2	9.2
GR 7 10.0 ppm	19.5	21.9	17.1	10.5
GR 24 0.01 ppm	10.8	28.7	0.7	10.2
GR 24 0.10 ppm	20.5	32.8	7.4	10.3
GR 24 1.00 ppm	21.0	25.3	16.4	15.2
LSD (0.05)	4.5	9.8	4.2	

† Numbers are means of four replicates. Each replication consisted of at least 100 seeds.

Table 4. 4. Time required for maximum germination of ***O. aegyptiaca*** seeds after treatment with the strigol anologs.

Treatment	Days after GR treatment 1	2	3	5	7	14	LSD (0.05)
	------------------ % germination ------------------						
Control	0.80	7.10	7.60	9.40	8.30	9.60	2.2
GR 7 0.001 ppm	0.90	41.3	54.5	67.5	70.6	77.3	6.0
GR 7 0.010 ppm	1.50	35.5	62.2	61.6	72.8	78.2	8.2
GR 7 0.100 ppm	2.00	45.8	48.8	72.4	73.9	73.4	9.3
GR 7 1.000 ppm	1.50	30.4	32.8	66.3	73.7	72.5	9.8
GR 24 0.001 ppm	4.90	43.3	71.1	72.7	70.0	71.8	13.0
GR 24 0.010 ppm	3.50	41.1	70.2	74.6	74.5	77.7	14.0
GR 24 0.100 ppm	5.40	29.5	61.4	72.8	75.0	74.4	11.7
GR 24 1.000 ppm	5.00	38.3	60.7	74.2	77.8	79.5	20.2
LSD (0.05)	3.0	15.60	10.30	7.30	8.60	8.90	

† Numbers are means of three replicates. Each replication consisted of about 200 seeds.

to induce germination. These results are in agreement with those obtained by Saghir (17), who reported that treatment of preconditioned *O. ramosa* seeds with GR 24 at 0.001 to 1.0 ppm resulted in maximum germination of about 90%, 9 to 12 days after treatment. Similar results were obtained with GR 7 by the same researcher in an earlier experiment. The slight difference in the time required to obtain maximum germination of broomrape seeds after treatment with the strigol analogs between the above results and those of Saghir (17) may be due to the difference in broomrape species used. Kasasian (10) reported that maximum germination of *O. aegyptiaca* occurred 5 days after applying a host root exudate to preconditioned seeds.

To study the effect of light on germination of *O. aegyptiaca*, two different seed lots (1974 and 1978) were used. The newer (1978) seeds showed a maximum germination of about 85% as compared to the older (1974) seeds which showed maximum germination of only about 40% (Table 4.5). The germination of newer seeds incubated in light during preconditioning or during strigol analog treatment was not significantly different from that of seeds incubated during either or both treatment period(s) in the dark. However preconditioning of older seeds in the dark gave significantly higher germination than preconditioning them in the light. Also, seeds kept in the dark during preconditioning and after treatment with the strigol analogs showed a significantly higher germination than seeds preconditioned in the dark but kept in the light after treatment with the strigol analogs.

There are conflicting reports in the literature on the effect of light on broomrape seed germination. Rangaswamy (16) reported that light completely inhibited the germination of *O. aegyptiaca* seeds, whereas Izard and Hitier (cited in 8) did not observe any difference in the germination of *O. ramosa* seeds incubated either in the light or the dark. Privat (15) made similar observations as the latter investigators with *O. hederae* seeds and concluded that light played little or no part in the germination potential. In these experiments, the presence of light during either germination phase of *O. aegyptiaca* lowered the percent germination of older but not newer seeds. This may be due to the reason that the older seeds had much lower germination potential than the newer seeds and that seeds become increasingly sensitive to

Table 4. 5. Effect of light and growth regulators on germination of ***O. aegyptiaca*** seeds.

Preconditioning†	Stimulant‡ treatment	GR 7 1 µM	GR 7 5 µM	GR 24 1 µM	GR 24 5 µM
		------------- % germination			---------------
Older seeds					
Light	Light	12.9	16.5	15.4	18.1
Dark	Light	30.2	24.7	12.6	21.7
Light	Dark	17.4	12.4	13.6	13.8
Dark	Dark	28.5	31.3	33.0	30.6
	LSD (0.05)	4.8	4.8	4.8	4.8
Newer seeds					
Light	Light	78.6	74.2	79.2	79.7
Dark	Light	72.8	76.4	75.7	71.5
Light	Dark	76.2	79.6	83.1	81.9
Dark	Dark	74.7	83.7	73.3	80.9
		(NS)*	(NS)	(NS)	(NS)

† Seeds were preconditioned in water.

‡ Preconditioned seeds were treated with growth regulators, GR 7 or GR 24, for 7 days before germination was counted.

* NS refers to no significant difference.

environment stress as they age (14). Therefore, it appears that light may act as one of the inhibitory factors in broomrape seed germination at least in older, less vigorous seeds.

O. aegyptiaca seeds preconditioned in phosphate buffers with pH ranging from 4.5 to 7.5 showed some spontaneous germination. Preconditioning of broomrape seeds at pH 4.5 induced significantly higher spontaneous germination than that obtained by preconditioning at higher pH (Table 4.6). Spontaneous germination decreased with increase in pH of the preconditioning solution. No spontaneous germination occurred when the seeds were preconditioned at pH 8.0.

O. aegyptiaca seeds treated with 1 ppm GR 24 after preconditioning in solutions ranging from pH 5.0 to 8.0 did not show a significant difference in percent germination (Table 4.6). It is interesting to note that although seeds preconditioned at pH 4.5 showed the highest spontaneous germination, they showed a significantly lower germination following treatment with 1 ppm GR 24 than seeds preconditioned at a higher pH and then treated with the strigol analog. The reason for lower percent germination at 4.5 is not known.

O. aegyptiaca seeds preconditioned in distilled water (pH 6.5) and then treated with 1 ppm GR 24 solutions with pH ranging from 4.5 to 8.0 showed varible germination (Table 4.7). Seeds treated with GR 24 at pH 4.5 or 6.5 showed the highest percent germination, whereas seeds treated with GR 24 at pH 8.0 showed very low germination. Cohn and Hughes (5) have reported that the activity of dormancy-breaking agents, such as azide, hydroxylamine, and cyanide, of dehulled red rice (*Oryza sativa* L.) was pH-dependent such that optimum pH values favoring formation of the uncharged chemical species resulted in the highest germination percentages. It is thus possible that high pH (8.0) may have affected the chemistry of GR 24, which resulted in its inactivation.

Interestingly, broomrape seeds preconditioned at pH 6.5 and then treated with a solution with pH 4.5 containing no strigol analogs showed about 57% germination which was not significantly different from percent germination obtained after treatment with GR 24 solution at a pH range of 5.0 to 6.0 or 7.0 to 7.5. As in the case of seeds treated with the solution of GR 24 at pH 8.0, germination of seeds preconditioned at pH 4.5 and then treated

Table 4. 6. Effect of pH on preconditioning of ***O. aegyptiaca*** seeds.

pH†	Without GR 24‡ treatment	With GR 24‡ treatment
	----------------- % germination	-----------------
4.5	17.7	66.0
5.0	13.5	75.9
5.5	11.9	75.4
6.0	5.1	72.5
6.5	3.3	75.4
7.0	4.2	73.9
7.5	1.8	74.2
8.0	0.0	75.5
LSD (0.05)	3.8	5.1

† pH of the preconditioning solution.

‡ pH of the postconditioning solution with or without GR 24 was adjusted to 6.5. Numbers are means of four replicates and each replication consisted of at least 100 seeds.

Table 4. 7. Effect of pH of the preconditioning or postconditioning solutions on germination of ***O. aegyptiaca*** seeds.

pH during preconditioning	pH during postconditioning	Germination†
		(%)
no GR 24	with GR 24	
6.5	4.5	70.6
6.5	5.0	54.3
6.5	5.5	42.7
6.5	6.0	53.5
6.5	6.5	71.2
6.5	7.0	63.2
6.5	7.5	61.5
6.5	8.0	3.9
no GR 24	no GR 24	
4.5	6.5	26.1
4.5	8.0	6.6
6.5	4.5	57.1
8.0	4.5	32.2
LSD (0.05)		7.1

† Numbers are means of four replicates. Each replication consisted of at least 100 seeds.

with a solution at pH 8.0 but without any strigol analogs was considerably lower than that of seeds treated with a solution of lower pH after preconditioning. Kasasian (10) observed that when *O. crenata* were preconditioned at pH 6 and then treated with a root exudate, percent germination was higher than when the seeds were preconditioned at pH 5. The above results do not show a significant effect of pH of the preconditioning solution on germination, except when the pH of the solution was as low as 4.5. Also it appears that incubation of broomrape seeds first at pH 6.5 and then pH 4.5 may induce spontaneous germination without the help of a germination stimulant.

From these results, it appears that the pH of the preconditioning solution does not affect the germination of broomrape seeds as much as the pH of the postconditioning solution. This may be due to the effect of pH on the activity of the germination stimulant. Also, it appears that the pH of the postconditioning solution may, somehow affect the spontaneous germination (i.e., germination in the absence of an external germination stimulant) of broomrape seeds. Kasasian (10) has reported that sorghum and broad bean root exudates were most active in inducing germination of *O. aegyptiaca* seeds at pH between 4 and 5 and between pH 6 and 7. Whitney (19) reported that changes in pH had a significant effect on the activity of broad bean extracts and that the activity of the extracts decreased slightly with the increase in pH of the medium from 6.3 to 7.5. The above results with a synthetic germination stimulant (GR 24) confirm these observations.

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5.0 EFFECT OF STRIGOL ANALOGS AND OTHER GROWTH REGULATORS ON GERMINATION OF BROOMRAPE SEEDS

5.1 INTRODUCTION

Broomrape seeds have the ability to remain viable in the soil for a number of years. For example, Kadry and Tewfic (15) reported that *O. crenata* Forsk. seeds could remain viable for more than 10 years in most climates. This long term viability is most pernicious, since the seeds can remain undetected in the soil until host plants are planted.

There have been conflicting reports in the literature on the dormancy and germination of broomrape seeds. It has now become clear, however, that broomrape seeds germinate in the presence of live hosts and some nonhosts or exudates from the roots of these plants. Recently, it has been discovered that live plants or their root exudates were not necessary in bringing about the germination of broomrape seeds, but a chemical present in root exudates

of suitable plants was sufficient to stimulate germination (3, 20). Cook et al., (5, 6) isolated a chemical from the roots of cotton (*Gossypium hirsutum* L.) that stimulated the germination of witchweed (*Striga* spp.) seeds (12). Appropriately, this chemical stimulant was called strigol. The characterization of the structure of strigol led to the synthesis of certain strigol analogs (5). Several of these strigol analogs were found to stimulate germination of broomrape seeds (13). Following these findings, an approach was formulated by which broomrape seed populations in the soil could be reduced by inducing "suicidal germination" (i.e. germination in the absence of host plants, which can lead to death of the germinating broomrape) with the help of synthetic growth regulators.

Ethylene has been shown to stimulate germination of many weed seeds including those of *Striga* (8, 9). Field studies indicated a 90% reduction in viable witchweed seeds following a single treatment with ethylene (9). Reports on the effect of ethylene on broomrape seed germination are inconsistent, however. Chun et al. (4) reported that soil-preconditioned broomrape seeds in humidified air-tight jars showed 17 times higher germination when exposed to ethylene than untreated seeds. Exudates from mung bean (*Vigna radiata* L.) roots did not improve germination of broomrape seeds exposed to ethylene, indicating that ethylene replaced the need for the exudates. Application of ethylene to broomrape-infested soil resulted in a decrease of broomrape attachment to tomato by about 60%. Higher rates of ethylene appeared to be inhibitory to broomrape seed germination (4). In other experiments, however, ethylene did not induce germination of preconditioned broomrape (*O. aegyptiaca* Pers.) seeds in the laboratory (16) and it had no effect in stimulating germination of broomrape seeds in the presence of a natural stimulant (7).

Besides strigol, strigol analogs, and ethylene, many other growth regulators have been observed to stimulate germination of broomrape seeds. Hiron (11) reported that gibberellic acid was effective in inducing germination of *O. ramosa* L. seeds, and Privat (18) found it stimulatory to germination of *O. hederae* seeds. Furthermore, Nash and Wilhelm (17) reported that, although *O. ludoviciana* Nutt. seeds germinated spontaneously, the germination percentage was considerably increased by gibberellic acid, and Abu-Shakra et al. (1) found

that *O. ramosa* seed pretreated with gibberellic acid (100 ppm) gave higher germination than seeds pretreated with water or host root exudates. Rangaswamy (19) added kinetin and yeast extract to the list of stimulatory substances for the germination of *O. ramosa* seeds.

The objectives of this investigation were to determine the effect of strigol analogs, ethephon, and gibberellic acid on *O. aegyptiaca* seed germination and to elucidate the processes involved in the germination stimulation of broomrape seeds by strigol analogs.

5.2 MATERIALS AND METHODS

5.2.1 Standard Germination Procedure

O. aegyptiaca seeds used in these experiments were obtained from broomrape-parasitized potato plants in the Shave Zion region of Israel in 1978. Dry broomrape seeds were surface sterilized with Sporicidin and preconditioned in 9-cm glass petri dishes on GFFP disks soaked with sterilized, distilled water or a growth regulator solution. All petri dishes were sealed with a strip of parafilm to prevent loss of moisture during preconditioning and kept in a plastic container wrapped in aluminum foil. The seeds were incubated in the dark at 23 to 25°C for 10 days.

After the preconditioning period, the GFFP disks were transferred to clean GFFP disks in sterilized petri dishes. The disks were supplied with 50 µl of distilled water containing 0.1% (v/v) DMSO or a growth regulator solution also containing 0.1% (v/v) DMSO. All procedures were carried out under physiologically "safe" green light and the seeds were incubated in the dark at 23 to 25°C for 1 week. Each petri dish contained four or eight GFFP disks and represented one treatment replicate. There were four or five replicates for each treatment in

a randomized complete block design. Germination of broomrape seeds was determined using a binocular microscope.

5.2.2 Effect of Ethephon on Broomrape Seed Germination

Ethephon (69% solution; Union Carbide, Raleigh, NC) was used as the source of ethylene. Ethephon solutions with concentrations ranging from 0.1 to 100 ppm were prepared in distilled water containing 0.1% (v/v) DMSO. For the preconditioning treatments, 3 ml ethephon solution were applied to each petri dish containing 25 GFFP disks with broomrape seeds. During the postconditioning treatment, 50 µl of ethephon solution was applied to each GFFP disk. The petri dishes were kept sealed with parafilm during preconditioning and postconditioning treatments to reduce the loss of gas or moisture. The treatments were replicated four times and each replication consisted of about 100 seeds.

5.2.3 Effect of Gibberellic Acid and Ancymidol on Broomrape Seed Germination

GA_3 (75% potassium salt) was used to study the effect of gibberellins on *O. aegyptiaca* germination. Dry surface-sterilized broomrape seeds were preconditioned in distilled water or tenfold dilutions of GA_3 within the concentration range of 0.001 to 1.0 mM. Preconditioned broomrape seeds were treated with 0.1% DMSO in distilled water, 5 µM GR 24 or GA_3 solution.

In order to test if the strigol analogs triggered gibberellin synthesis in broomrape seeds, ancymidol [α-cyclopropyl-α- (4-methoxyphenyl)-5-pyrimidinemethanol] was used alone and in combination with the strigol analogs to inhibit endogenous gibberellin synthesis. In one experiment, broomrape seeds were preconditioned in distilled water for 10 days at 23 to 25°C.

Following the preconditioning period, the seeds were treated with 0.1 to 1.0 mM ancymidol solution prepared in 0.1% DMSO applied alone or in mixture with 5 µM GR 24 or with 5 µM GR 24 and 0.01 to 1.0 mM GA_3 solutions. GR 24 and GA_3 at various concentrations applied alone were also included in the treatments.

In another experiment involving ancymidol, *O. aegyptiaca* seeds were preconditioned in either distilled water or ancymidol solutions. Preconditioned broomrape seeds were treated with either distilled water (0.1% DMSO solution) or with ancymidol, GR 24 and/or GA_3 applied sequentially with a difference of 1 day. Each time a treatment was applied, the GFFP disks containing the broomrape seeds were removed from the petri dishes, dabbed on dry filter papers to remove extra moisture and placed on new GFFP disks soaked with the test solutions in clean petri dishes. This procedure was used to apply the sequential treatments with ancymidol, GR 24 and/or GA_3 also. Percent germination was obtained 1 week after the last sequential treatment. Each treatment was replicated four times in a completely randomized block design. Means were compared using the least significant difference (LSD) test at the 5% level of significance.

5.3 RESULTS AND DISCUSSION

5.3.1 Effect of Ethephon on Broomrape Seed Germination

O. aegyptiaca seeds incubated in distilled water alone during both preconditioning and postconditioning phases of germination showed a spontaneous germination of 7.3%. In contrast, broomrape seeds preconditioned in distilled water and then treated with GR 24 showed a maximum germination of about 76% (Table 5.1). Incubation of broomrape seeds in

Table 5. 1. Effect of ethephon and GR 24 on germination of ***O. aegyptiaca*** seeds.

Preconditioning treatment		Postconditioning treatment	Germination†
			(%)
Water		Water	7.3
Water		GR 24 5 μM	76.5
Ethephon	0.1 ppm	Ethephon 0.1 ppm	3.3
Ethephon	1.0 ppm	Ethephon 1.0 ppm	10.8
Ethephon	10.0 ppm	Ethephon 10.0 ppm	9.7
Ethephon	100.0 ppm	Ethephon 100.0 ppm	15.4
Water		Ethephon 0.1 ppm	26.8
Water		Ethephon 1.0 ppm	38.7
Water		Ethephon 10.0 ppm	34.1
Water		Ethephon 100.0 ppm	52.8
Ethephon	0.1 ppm	GR 24 5 μM	77.4
Ethephon	1.0 ppm	GR 24 5 μM	67.1
Ethephon	10.0 ppm	GR 24 5 μM	75.3
Ethephon	100.0 ppm	GR 24 5 μM	52.9
LSD (.05)			3.8

† Numbers are means of four replicates. Each replication consisted of about 100 seeds.

ethephon alone during either or both phases of germination showed significantly lower germination than incubation of seeds in GR 24 during postconditioning.

Incubation of broomrape seeds in ethephon during both phases of germination showed a maximum germination of about 15%. However, when the seeds were preconditioned in water and then treated with ethephon, they showed a maximum germination of about 53%. Thus, it appears that treatment of preconditioned broomrape seeds with ethylene is more effective in inducing germination than treatment of nonpreconditioned seeds. Preconditioning of seeds in ethephon followed by treatment with GR 24 gave germination equivalent to that obtained with GR 24 applied to water-preconditioned broomrape seeds.

These results are in contrast with those of Kasasian (16), who observed no germination of preconditioned *O. aegyptiaca* seeds with ethephon within a concentration range of 0.1 to 100 ppm. Ethylene being a gas could easily escape from the petri dishes during the treatment period in an unsealed system. The details of the procedure used by Kasasian were not available from the report. Chun et al. (4) has reported that ethephon applied to soil preconditioned *O. ramosa* seeds in humidified air-tight jars stimulated germination 17 fold more than the control.

5.3.2 Effect of Gibberellic Acid and Ancymidol on Broomrape Seed Germination

As noted earlier, *O. aegyptiaca* seeds showed about 7% spontaneous germination. Application of gibberellic acid to broomrape seeds improved germination significantly over the control. Treatment of water-preconditioned broomrape seeds with 1 mM gibberellic acid resulted in about 69% germination, which was not significantly different from that obtained with 5 μM GR 24 (Table 5.2). Lower concentrations of gibberellic acid were less effective in inducing germination. There was no difference in germination when the seeds were treated

Table 5. 2. Effect of gibberellic acid on germination of ***O. aegyptiaca*** seeds.

Preconditioning treatment	Postconditioning treatment	Germination†
		(%)
Water	Water	7.20
Water	GR 24 5 µM	73.9
GA_3 0.001 mM	Water	5.00
GA_3 0.010 mM	Water	11.0
GA_3 0.100 mM	Water	40.1
Water	GA_3 0.001 mM	5.00
Water	GA_3 0.010 mM	11.4
Water	GA_3 0.100 mM	34.0
Water	GA_3 1.000 mM	68.5
GA_3 0.001 mM	GA_3 0.001 mM	3.00
GA_3 0.010 mM	GA_3 0.010 mM	17.6
GA_3 0.100 mM	GA_3 0.100 mM	35.3
GA_3 1.000 mM	GA_3 1.000 mM	62.5
	LSD (.05)	6.30

† Numbers are means of four replicates. Each replication consisted of about 100 seeds.

with gibberellic acid either during preconditioning or during postconditioning or during both phases of germination.

Reports on the effect of gibberellic acid on broomrape seed germination have been quite variable. Kasasian (16) reported that gibberellic acid at 100 ppm induced only 5% germination of nonpreconditioned seeds and about 8% germination of water preconditioned seeds of *O. aegyptiaca*. Hiron (11) observed about 13% germination of *O. crenata* seeds preconditioned in 20 ppm gibberellic acid and Nash and Wilhelm (17) observed about 17% germination of *O. ramosa* seeds preconditioned in 100 ppm gibberellic acid. A considerable increase in germination was observed, however when the gibberellin-pretreated seeds were incubated with a stimulant, such as exudates from faba bean (*Vica faba* L.) (11) or flax (*Linum usitatissimum* L.) (1, 10).

Brown (3) suggested that a certain critical level of the stimulant must be established in the seed before germination can occur. This critical quantity of the stimulant is contributed in part by the seed itself and in part by an external supply. During preconditioning there is at first a gradual accumulation of the stimulant until a maximum quantity is formed and then there is a decrease of the accumulated reserve. It has also been suggested that as preconditioning proceeds, the increase in germination with a standard concentration of the stimulant is the result of a closely related substance in the seed. The amount of stimulant in the individual seeds at any stage of preconditioning varies considerably, and at all stages the variation involves some seeds that can provide the whole of their own stimulant requirement. The seeds that contain sufficient quantity of their own stimulant germinate spontaneously. The number of such seeds in a population is small.

Dormant seeds of broomrape contain a very low concentration of gibberellins (1) and application of exogenous gibberellins can induce germination of broomrape seeds. Abdel Halim et al. (2) found that maximum germination of broomrape takes place in the vegetative phase of the host when gibberellin production is highest. Hiron (11) distinguished between an endogenous stimulant, present at a concentration below that required for triggering germination, and the host derived external stimulant. The latter may well act with the former

and the stimulatory effects observed with gibberellic acid may well be due to the effects it has on the concentration or activity of the endogeneous stimulant.

Gibberellins have been shown to have a mobilizing effect on barley (*Hordeum vulgare* L.) endosperm (14, 21). Gibberellic acid affects the liberation of sugars and enzymes in carbohydrate metabolism of barley endosperm, and carbohydrate metabolism during seed germination is controlled by an embryonic source of gibberellins. Thus, it appears that gibberellins may be partially responsible for controlling germination of broomrape seeds and that GR 24 may act by inducing endogenous gibberellin synthesis which, in turn, may stimulate broomrape seed germination. In order to test this hypothesis, a gibberellin synthesis inhibitor, ancymidol, was applied to broomrape seeds in combination with GR 24.

Gibberellic acid at 1 mM concentration applied alone to water preconditioned broomrape seeds induced about 78% germination of *O. aegyptiaca*, which was equivalent to that obtained with 5 μM GR 24. Ancymidol applied alone appeared to stimulate germination of broomrape seeds at the lowest concentration (0.01 mM), but not at higher concentrations (Table 5.3). When ancymidol was applied with GR 24, broomrape seed germination was significantly lower than that obtained with GR 24 alone. At the highest ancymidol concentration (1.0 mM) applied in combination with GR 24, no germination was obtained. Addition of gibberellic acid to the mixture of ancymidol and GR 24 did not increase germination over that obtained with the mixture of ancymidol and GR 24. This may be due to the fact that the highest concentration of ancymidol used in this experiment was toxic to broomrape seeds and the concentration of gibberellic acid used in combination with lower ancymidol concentrations was too low to bring any recovery in germination. It is also possible that ancymidol, GR 24, and/or gibberellic acid may have chemically reacted with each other before entry into the seeds, which may have resulted in a decrease of the activity of any one or all three compounds.

In order to avoid any possible interaction of compounds before application to seeds, ancymidol, GR 24, and gibberellic acid were applied sequentially with an interval of 1 day between applications. *O. aegyptiaca* seeds in this experiment showed about 13% spontaneous

Table 5. 3. Effect of ancymidol on germination stimulation of ***O. aegyptiaca*** seeds by gibberellic acid and GR 24.

Treatment†		Germination‡
		(%)
Control		7.6
GR 24 5 μM		75.3
GA_3 0.01 mM		42.9
GA_3 0.10 mM		59.7
GA_3 1.00 mM		77.7
Ancymidol 0.01 mM		26.1
Ancymidol 0.10 mM		5.0
Ancymidol 1.00 mM		0.0
Ancymidol 0.01 mM + GR 24 (5 μM)		66.6
Ancymidol 0.10 mM + GR 24 (5 μM)		59.7
Ancymidol 1.00 mM + GR 24 (5 μM)		0.0
Ancymidol 0.01 mM + GR 24 (5 μM) + GA_3 (0.01 mM)		65.7
Ancymidol 0.10 mM + GR 24 (5 μM) + GA_3 (0.10 mM)		59.7
Ancymidol 1.00 mM + GR 24 (5 μM) + GA_3 (1.00 mM)		0.0
	LSD (.05)	6.3

† All treatments were applied to water preconditioned broomrape seeds. Treatments involving two or three compounds were applied as tank mixtures.

‡ Numbers are means of four replicates. Each replication consisted of about 100 seeds.

germination. Treatment of water-preconditioned seeds with 5 μM GR 24 resulted in about 48% germination (Table 5.4). Ancymidol applied alone to water-preconditioned broomrape seeds showed about 20% germination at 0.01 mM and less than 1% germination at higher concentrations. Application of 5 μM GR 24 1 day after treatment of water-preconditioned broomrape seeds with ancymidol increased germination. However, maximum germination obtained after treatment with ancymidol and GR 24 was considerably lower than that obtained after treatment with GR 24 alone. Application of GR 24 to broomrape seeds preconditioned in ancymidol at the lowest concentration gave germination equivalent to that obtained with the application of GR 24 to water-preconditioned seeds. Preconditioning of broomrape seeds in 0.1 mM or higher ancymidol concentration inhibited germination significantly. When gibberellic acid was applied sequentially along with ancymidol and GR 24 to water-preconditioned seeds, germination was as high as that obtained after application of GR 24 alone to water-preconditioned seeds.

These results indicate that ancymidol, an inhibitor of endogenous gibberellin synthesis, is inhibitory to broomrape seed germination. The inhibitory action of ancymidol was more pronounced when it was applied during postconditioning than during preconditioning. These results tend to support the hypothesis that GR 24 applied to preconditioned broomrape seeds stimulates germination by inducing endogenous gibberellin synthesis, which is inhibited in the presence of ancymidol applied along with GR 24. The increase in germination of broomrape seeds when gibberellic acid was supplied to ancymidol and GR 24 treated seeds further supported the hypothesis by indicating that broomrape seed germination was inhibited in the presence of ancymidol for lack of gibberellins.

Hiron (11) had suggested that under conditions favorable to germination, *Orobanche* seeds metabolically produce an endogenous stimulant (ES) from a precursor (P). The ratio of P and ES could be affected by an external stimuli. The concentration of ES in broomrape seeds brought about by moisture conditioning alone, is generally insufficient to affect germination. Under the influence of gibberellic acid or any other stimulant, the concentration of ES in broomrape seeds may be raised sufficiently above its normal level to induce germination.

Table 5. 4. Effect of ancymidol on germination stimulation of ***O. aegyptiaca*** seeds by gibberellic acid and GR 24.

Treatment†	Germination‡
	(%)
Control	13.4
GR 24 5 μM	48.3
Ancymidol 0.01 mM (postcond.)	20.5
Ancymidol 0.10 mM (postcond.)	0.4
Ancymidol 1.00 mM (postcond.)	0.3
Ancymidol 0.01 mM + GR 24 5 μM (postcond. seq.)	34.2
Ancymidol 0.10 mM + GR 24 5 μM (postcond. seq.)	5.4
Ancymidol 1.00 mM + GR 24 5 μM (postcond. seq.)	0.4
Ancymidol 0.01 mM (precond.) + GR 24 5 μM (postcond.)	52.7
Ancymidol 0.10 mM (precond.) + GR 24 5 μM (postcond.)	11.8
Ancymidol 1.00 mM (precond.) + GR 24 5 μM (postcond.)	1.3
Ancymidol 0.01 mM + GR 24 5 μM + GA_3 1.0 mM (postcond. seq.)	47.1
Ancymidol 0.10 mM + GR 24 5 μM + GA_3 1.0 mM (postcond. seq.)	52.0
Ancymidol 1.00 mM + GR 24 5 μM + GA_3 1.0 mM (postcond. seq.)	12.0
LSD (.05)	7.0

† Treatments were applied to water-preconditioned or ancymidol preconditioned broomrape seeds. In treatments involving two or three compounds applied during post-conditioning, the compounds were applied in sequence with an interval of 1 day.

‡ Numbers are means of four replicates. Each replication consisted of about 100 seeds.

The identity of endogenous stimulant is not known. In the presence of strigol analogs, broomrape seeds accumulate a sufficient concentration of an endogenous stimulant and germinate. Hiron (11) suggested the possibility that the external stimulant and the endogenous stimulant (ES) are the same or very similar compounds in which case their combined effect would simply be additive. From these results, it appears that the strigol analogs do not substitute the endogenous stimulant, but they probably induce the synthesis of an endogenous stimulant in broomrape seeds. From preliminary investigations, it appears that the endogenous stimulant may be gibberellins. More detailed investigations will be required, however, to establish clearly the identity of the endogenous stimulant in broomrape seeds.

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6.0 SELECTIVE CONTROL OF BROOMRAPE IN CROPS BY FOLIARLY APPLIED HERBICIDES

6.1 INTRODUCTION

Broomrapes (*Orobanche* spp.) cause severe reductions in the yield of many important crops grown in the semi-arid regions of the world. A review of the literature on broomrape control indicates that there is no consistently effective and economical method, especially after the parasite has attached itself to crop plants. Soil fumigation with methyl bromide to destroy broomrape seeds is the only effective, but expensive, method available to control this parasite at present (37, 38, 39).

Selective control of broomrape is very difficult due to the close ecological and physiological relationship that exists between the host and the parasite. Literally hundreds of preplant-incorporated, preemergence, and postemergence herbicides have been investigated (16, 18, 20, 21, 22, 29, 30). Results in advanced field testing with these herbicides, however, have been largely disappointing.

Of all the herbicides tested, glyphosate [*N*-(phosphonomethyl) glycine] used as a systemic, postemergence spray to the host crop, currently shows most promise for widescale development. It is a relatively nonselective herbicide (1, 10, 34); nevertheless, some interesting selectivities have been demonstrated in certain crops, including some that are attacked by broomrape species (13, 17, 19, 20, 21, 22, 24, 27, 31, 32). Thus far, most success has been achieved with glyphosate on broad bean (*Vicia faba* L.). Recently, Lolas (23) has demonstrated that glyphosate can also be used successfully to control broomrape in certain varieties of tobacco (*Nicotiana tabacum* L.).

The effectiveness of glyphosate as a foliar application for *O. crenata* Forsk. control in broad bean was first demonstrated by Kasasian (17). The rate of 200 g/ha provided complete control of the parasite and sufficient safety margin indicating the relative resistance of broad bean to the herbicide. However, the rate of glyphosate necessary for control of other broomrape species on other crops grown during different seasons of the year have been less encouraging. Jacobsohn and Kelman (13) observed that December-sown broad bean and pea (*Pisum sativum* L.), both parasitized by *O. crenata*, showed different susceptibility to glyphosate. Broad bean proved to be resistant to two sprays of 150 g/ha glyphosate; however, pea was very sensitive to similar treatment. Tomato (*Lycopersicon esculentum* Mill.) also has shown considerable sensitivity to glyphosate; although varietal differences in sensitivity have been observed (9, 12).

Translocation of glyphosate to underground propagules of perennial species prevents regrowth from these sites and results in their subsequent destruction (1). Based on indirect evidence from field studies, it is thought that, when glyphosate is applied at considerably reduced rates (1/10th to 1/20th of recommended herbicidal rates), it may also be transported through a host plant and into the growing broomrape, thereby bringing about the suppression and/or control of the parasite (13, 17, 24, 27, 31).

Recently, several reports have indicated that glyphosate is exuded from the roots of treated plants into the surrounding medium (4, 5, 6, 26) from which it may be taken up by adjacent plants (5, 26). It is thus possible that glyphosate applied to host plants for broomrape

control may be exuded in small quantities into the rhizosphere of the roots where it affects germinated seeds of broomrape.

2,4-DB [4-(2,4-dichlorophenoxy) butyric acid] is a selective herbicide for broadleaf weed control in leguminous crops. The selectivity of plants to 2,4-DB is due to differential metabolism in leguminous crops and susceptible weeds. Few reports are available in the literature on the use of 2,4-DB for broomrape control in leguminous crops.

The objectives of these investigations were to evaluate the efficacy of single and multiple applications of glyphosate on tomato and that of a single application of 2,4-DB on peanut (*Arachis hypogaea* L.) and alfalfa (*Medicago sativa* L.) for broomrape control in the greenhouse and to determine the translocation and metabolism behavior of glyphosate in broomrape infested tomato plants. The effect of very low concentrations of glyphosate on germination of broomrape seeds was also evaluated.

6.2 MATERIALS AND METHODS

6.2.1 Efficacy of Glyphosate and 2,4-DB for Broomrape Control

The procedures used to grow tomato, peanut, and alfalfa plants were similar to that used to study the potential of broomrape species to parasitize different crops. Crop plants at the two-true-leaf stage were transplanted in 15-cm diameter plastic pots containing a potting medium. The potting medium consisted of equal volumes of clay loam, sand, and Weblite (expanded shale). Plants were inoculated with broomrape seeds (*O. aegyptiaca* Pers. obtained from the Massua region of Israel in 1974) at the time of transplanting by dispersing about 5 mg of the seeds directly on the roots of the crop plants. There were two plants each of tomato and alfalfa in each pot. Only one peanut plant was transplanted into each pot. The

commercial formulation of glyphosate (478 g/L Roundup®) at rates ranging from 25 to 75 g (a.i.)/ha in a spray volume equivalent to 250L/ha was applied to tomato plants using a laboratory moving-belt or a small plot hand-held sprayer. The plants were sprayed at 2, 4, and/or 6 weeks after transplanting. The surface of the potting medium was covered with paper towels before spraying the broomrape plants directly. Plants were harvested 8 weeks after transplanting and observations on tomato shoot height and root and shoot fresh weights (FW) and broomrape number of infections, shoot height above soil surface, and shoot fresh weight were recorded.

The commercial formulation of 2,4-DB (239 g/L. Butyrac®) at 0.1 or 0.2 kg/ha was applied to peanut and alfalfa plants 4 or 6 weeks after transplanting. The herbicide applications were made in a spray volume equivalent to 250 L/ha using a small plot hand-held sprayer as with glyphosate, the surface of the potting medium was covered with paper towels to prevent the herbicide from reaching the broomrape directly. Plants were harvested 9 weeks after transplanting and observations on peanut and alfalfa shoot height and root and shoot fresh weights (FW) and broomrape number of infections, shoot height above soil surface, and shoot fresh weight were recorded. All treatments were replicated six times, and the data were analyzed statistically using the least significant difference (LSD) test at the 5% level of significance.

6.2.2 Translocation and Metabolism of Glyphosate

6.2.2.1 Plant Material

The translocation and metabolism of glyphosate were studied in broomrape-infested tomato plants in the greenhouse. Tomato plants at the two-true-leaf-stage were transplanted into 15-cm diameter plastic pots containing the same potting medium as in the above

experiments. The plants were inoculated with 5 mg broomrape (*O. aegyptiaca*) seeds at the time of transplanting. The pots were watered as needed to maintain normal plant growth. The temperature in the greenhouse ranged from 22 to 30°C and the day length was 16 h.

6.2.2.2 *Herbicide Application Procedure*

^{14}C-glyphosate (specific activity 1.97 mCi/mmol) supplied by Monsanto, Chemical company was dissolved in distilled water. The herbicide was formulated into the isopropylamine salt by adding an equimolar amount of isopropylamine. To this mixture, a nonionic surfactant MON 0818 was added at a concentration of 0.1%. The total radioactivity present in the solution was 10 μCi. The stock solution was kept sealed and refrigerated when not in use.

Tomato plants were treated when the broomrape plants had just emerged (i.e. about 6 weeks after transplanting). Radiolabeled herbicide solution was applied in 10 μl droplets to twelve mature leaflets on each tomato plant using an Oxford automatic pipetter. The droplets were applied near the midvein in the middle of the leaflets. Each plant received a total of 0.25 μCi radioactivity in 120 μl volume. The plants were harvested 3 and 7 days after herbicide application.

6.2.2.3 *Herbicide Detection and Identification Procedure*

Harvested tomato plants were separated into the treated leaves, the growing point, the shoot (remainder of the foliage and stem), and the roots along with the attached broomrape plants. The sectioned plants were mounted, pressed and dried in an oven at 70°C for 48 h. Comparative uptake and translocation studies were performed using the autoradiographic methods of Crafts and Yamaguchi (7) and standard radioassay techniques. Dry tomato plants were exposed to X-ray film for 3 to 4 weeks.

The plants used for autoradiography were subsequently used for the quantitative analysis of radioactivity by liquid scintillation counting. The procedures of Gottrup et al. (11) were used for extracting the radioactive glyphosate from plant tissues. The dried plant material was ground in a Wiley mill and shaken for 4 h in a solution containing 100 ml distilled water, 0.5 ml solution of ethyl mercurithiosalicylate (Thimerosal), and 0.5 ml of 0.2 M ammonium carbonate. The contents of the flasks were filtered, the shaking process was repeated with fresh solution and the two filtrates were combined and freeze dried. The dry residue was redissolved in 2 ml distilled water. A 50 µl volume of this aliquot was pipetted into scintillation vials and 15 ml of a commercially prepared scintillation cocktail (Aquasol) was added. Radioactivity was determined on a Beckman liquid scintillation counter. Four plants, each representing one replicate for each day of harvesting, were used for determining the radioactivity.

The aliquots used for liquid scintillation counting were also used for metabolism studies. Thin layer chromatography (TLC) was used to analyze the extracts for glyphosate according to the method described by Sprankle et al. (35). A 50 µl volume of the aliquots was spotted onto cellulose TLC plates (250 µm thick MN 300 cellulose, from Analtech, Inc., Newark, Del.). The plates were developed for 15 cm in ethanol : water : 15 N ammonium hydroxide : 17 N acetic acid : trichloroacetic acid (55 : 35 : 2 : 5 : 2 : 3 : 5 :: v : v : v : v : w). When developed, the plates were dried and divided into fifteen 1-cm bands. Each band was scraped into a scintillation vial containing 15 ml Aquasol. The vials were shaken vigorously and cooled prior to counting in a liquid scintillation counter. From this, Rf values were calculated for each sample in which ^{14}C-activity was detected, and these values were compared to those quoted by Sprankle et al. (35) for glyphosate and several of its metabolites. As a check, ^{14}C-glyphosate from the stock solution was also included in the analysis.

6.2.3 Effect of Glyphosate on Germination of Broomrape Seeds

Surface-sterilized *O. aegyptiaca* seeds (obtained from the Shave Zion region of Israel in 1978) were preconditioned on GFFP disks placed on a single sheet of Whatman 5 filter paper in glass petri dishes. Each petri dish was given 3 ml distilled water or a glyphosate solution with concentration ranging from 0.01 to 10 mM. Glyphosate solutions were prepared by dissolving measured amounts of the commercial formulation of glyphosate in distilled water. The seeds were incubated at 22 to 25°C in the dark for 10 days.

After the preconditioning period, GFFP disks containing the broomrape seeds were transferred to clean petri dishes and retreated with distilled water or the glyphosate solutions. Half of the petri dishes with seeds treated with glyphosate were also treated with 5 µM GR 24 solution applied sequentially 1 day after glyphosate application. The seeds were incubated at 22 to 25°C in the dark for 1 week before germination was counted.

6.3 *RESULTS AND DISCUSSION*

6.3.1 Efficacy of Glyphosate and 2,4-DB for Selective Control of Broomrape in Crops

The efficacy of low rates of glyphosate and 2,4-DB was tested on tomato and peanut plants, respectively, in the greenhouse. Tomato plants not inoculated with broomrape were damaged significantly by 75 g/ha glyphosate applied 6 weeks after transplanting (Table 6.1). The lower rate (37.5 g/ha) of the herbicide did not seem to have any deleterious effect on tomato plants. Plant height and shoot and root fresh weights of untreated control plants

Table 6. 1. Effect of low rates of glyphosate on growth of 'Rutgers' tomato plants.

Treatment	Rate	Plant height	Shoot† FW	Root† FW
	(g/ha)	(cm)	(g)	(g)
Noninoculated				
Control		30.3	3.5	0.8
Glyphosate	37.5	32.6	3.9	0.8
Glyphosate	75.0	24.0	2.3	0.5
LSD (0.05)		2.7	0.6	0.2
Broomrape-infected				
Control		13.9	1.4	0.5
Glyphosate	37.5	13.2	1.2	0.4
Glyphosate	75.0	14.9	1.0	0.4
LSD (0.05)		2.7	0.6	0.2

† Numbers are means of six replicates. Each replication consisted of two plants.

infected with broomrape were considerably lower than those of noninoculated tomato plants. The effect of glyphosate at either rate was not apparent on the growth of tomato plants infected with broomrape.

The higher rate (75 g/ha) of glyphosate resulted in a significantly fewer infections and lower shoot fresh weights of broomrape plants growing on tomato plants (Table 6.2). Shoot height of broomrape plants treated with 75 g/ha glyphosate also appeared to be lower than in the control, but this difference was not statistically significant. The lower rate of the herbicide did not have any significant effect on the parasitism and growth of broomrape plants on tomato.

The higher rate of glyphosate, although effective in controlling broomrape in tomato, resulted in some injury to tomato. Crop plants treated with 75 g/ha showed typical glyphosate injury symptoms, which included bleaching of the tissue in the apical meristem. The lower rate of glyphosate did not result in any injury to tomato plants, but did not appear to be sufficient to control broomrape either. Based on these results, another experiment was conducted in the greenhouse that included single and multiple applications of glyphosate at very low rates on tomato plants.

Single applications of glyphosate at 37.5 g/ha applied 6 weeks after transplanting appeared to enhance the growth of tomato plants. Applications at 2 or 4 weeks after transplanting did not affect the growth of tomato plants significantly (Table 6.3). No injury symptoms were visible on the foliage of tomato plants. Glyphosate applied at 75 g/ha once at 4 or 6 weeks after transplanting appeared to inhibit the growth of tomato plants, although no statistically significant differences were observed between treated and control plants in shoot height and shoot fresh weight. Root fresh weight of tomato plants treated at 4 weeks after transplanting, however, was significantly lower than in the control.

Glyphosate applied twice at 37.5 g/ha either at 2 and 4 weeks or at 4 and 6 weeks after transplanting did not affect the shoot growth, but inhibited the root growth of tomato plants significantly. When glyphosate at 25 g/ha was applied at 2, 4, and 6 weeks after transplanting, there was no effect of the herbicide on shoot or root growth.

Table 6. 2. Effect of glyphosate on broomrape growth following application of the herbicide to tomato foliage.

Treatment	Rate	Infections† per plant	Shoot height	Shoot† FW
	(g/ha)		(cm)	(g)
Broomrape-infected Control		2.1	3.6	1.2
Glyphosate	37.5	2.2	3.4	0.7
Glyphosate	75.0	0.6	0.9	0.1
LSD (0.05)		1.0	3.0	0.6

† Numbers are means of six replicates. Each replication consisted of two plants.

Table 6. 3. Effect of single and multiple applications of glyphosate on growth of 'Rutgers' tomato plants.

Treatment†	Rate	Shoot‡ height	Shoot‡ FW	Root‡ FW
	(g/ha)	(cm)	(g)	(g)
Untreated control		14.7 bc	1.2 b	1.6 ab
Glyphosate (2 weeks)	37.5	16.0 bc	1.3 ab	1.4 ab
Glyphosate (4 weeks)	37.5	16.3 ab	1.3 ab	1.2 bcd
Glyphosate (6 weeks)	37.5	18.5 a	1.8 a	1.8 a
Glyphosate (4 weeks)	75.0	13.6 c	0.8 b	0.6 e
Glyphosate (6 weeks)	75.0	14.7 bc	0.9 b	1.3 abc
Glyphosate (2+4 wks)	37.5+37.5	13.8 bc	0.9 b	0.7 de
Glyphosate (2+6 wks)	37.5+37.5	14.3 bc	1.0 b	0.9 cde
Glyphosate (2+4+6 wks)	25+25+25	14.3 bc	1.3 ab	1.3 abc

† Herbicide applications were made 2, 4, and/or 6 weeks after transplanting tomato plants.

‡ Means in the same column followed by the same letter are not significantly different according to Duncan's Multiple Range Test at $P \leq 0.05$.

The mean number of broomrape infections on untreated tomato plants was 3.8. (Table 6.4). One application of glyphosate at 37.5 g/ha at 2, 4 or 6 weeks after transplanting did not reduce the number of broomrape infections, but inhibited the elongation of broomrape shoots significantly, especially following earlier applications at 2 or 4 weeks after transplanting. Shoot weight of broomrape was not affected by a single application of glyphosate at 37.5 g/ha. Surprisingly, the higher rate of glyphosate (75 g/ha) in this experiment did not affect the growth of broomrape plants treated with the herbicide. In the experiment conducted earlier in the greenhouse, 75 g/ha glyphosate had proved injurious to both tomato and broomrape plants. One possible reason for this inconsistency in results is that the two experiments were conducted at different times of the year. The first experiment was conducted during the summer of 1986, when the temperature in the greenhouse reached up to 35°C during the day. The second experiment was conducted during the spring of 1987, when the maximum temperature in the greenhouse was lower than 30°C. It is known that higher temperatures can increase the activity of glyphosate on plants (3).

Glyphosate applied twice at 37.5 g/ha either at 2 and 4 weeks or at 4 and 6 weeks after transplanting also did not affect the growth of plants. When the herbicide was applied thrice at 25 g/ha at intervals of 2 weeks, elongation of broomrape shoots was significantly inhibited, but the mean fresh weight of broomrape shoots was not affected. There was no difference in the number of broomrape infections on tomato plants with any of the treatments.

Glyphosate at low rates (60 to 120 g/ha) is reportedly very effective in controlling broomrape (13, 17, 19, 31, 32). From the above results, it appears that glyphosate at 37.5 g/ha applied once is not effective in controlling broomrape. The higher rate (75 g/ha) was effective in suppressing the growth of broomrape, but it was unsafe on tomato plants. Further research using multiple applications of low rates of glyphosate (25 g/ha to 50 g/ha) is therefore required to find a more useful way of using glyphosate for broomrape control in tomato. Some researchers, however, have indicated that due to the high sensitivity of tomato plants, the chances of glyphosate to be eventually used for broomrape control in this crop are very small (12, 15). With the discovery of a glyphosate-resistant gene in a genetically modified strain of

Table 6. 4. Effect of glyphosate on growth of broomrape following single and multiple applications of the herbicide on tomato plants.

Treatment†	Rate	Infections‡ per plant	Shoot‡ height	Shoot‡ FW
	(g/ha)		(cm)	(g)
Untreated control		3.8 ab	5.0 a	0.3 b
Glyphosate (2 weeks)	37.5	5.3 a	2.5 bcd	0.4 ab
Glyphosate (4 weeks)	37.5	3.7 ab	1.6 cd	0.2 b
Glyphosate (6 weeks)	37.5	3.4 a	3.2 abc	0.3 b
Glyphosate (4 weeks)	75.0	3.3 b	2.8 abc	0.3 b
Glyphosate (6 weeks)	75.0	3.3 b	1.8 bcd	0.3 b
Glyphosate (2+4 wks)	37.5+37.5	3.0 b	4.6 ab	0.4 ab
Glyphosate (2+6 wks)	37.5+37.5	3.4 b	3.5 abc	0.6 a
Glyphosate (2+4+6 wks)	25+25+25	3.7 ab	0.0 d	0.4 ab

† Herbicide applications were made 2, 4, and/or 6 weeks after transplanting tomato plants.

‡ Means in the same column followed by the same letter are not significantly different according to Duncan's Multiple Range Test at $P \leq 0.05$.

a bacterium, *Salmonella typhimurium* (2), which has been introduced by genetic engineering techniques into tobacco, tomato, and some other crops, there is hope that glyphosate can be safely used for broomrape control in these crops. Such genetically engineered crop plants are expected to become commercially available in the early 1990's (36).

Peanut plants treated with 2,4-DB 2 weeks after transplanting did not show any significant response (Table 6.5). Application of the herbicide at 0.2 kg/ha 4 weeks after transplanting resulted in significantly lower shoot fresh weight of peanut plants as compared to untreated control plants.

Nearly all peanut plants inoculated with broomrape became infected in this experiment. The mean number of broomrape infections on each peanut plant was 2.4 (Table 6.6). There was, however, no significant difference in the growth of noninoculated and broomrape-infected peanut plants.

Application of 2,4-DB at either rate 2 or 4 weeks after transplanting reduced parasitism of peanut plants by broomrape significantly as evidenced by the lower number of infections and broomrape shoot height (Table 6.6). Early application of 2,4-DB at 2 weeks after transplanting appeared to be more effective in reducing the number of broomrape infections on crop plants than did the later application. There was no significant effect of 2,4-DB applied at two different rates, except in the case of shoot height, where the higher rate appeared to be more effective than the lower rate in inhibiting shoot elongation. Shoot fresh weight of broomrape plants was not significantly affected by any of the treatments.

Few reports exist in the literature on the use 2,4-DB for broomrape control. The above results indicate that although 2,4-DB is required at much higher rates than glyphosate, it can be used to control broomrape in leguminous crops. However, further testing with this herbicide, especially in the field, is required before its efficacy for broomrape control can be fully known.

Table 6. 5. Effect of 2,4-DB on growth of peanut plants.

Treatment†	Rate	Plant height	Shoot‡ FW	Root FW
	(kg/ha)	(cm)	(g)	(g)
Noninoculated control		21.32	9.20 b	5.8
Broomrape-infected control		23.60	9.00 b	7.18
2,4-DB (4 weeks)	0.1	23.94	9.19 b	4.87
2,4-DB (4 weeks)	0.2	23.79	9.08 b	4.83
2,4-DB (6 weeks)	0.1	23.95	7.60 ab	4.78
2,4-DB (6 weeks)	0.2	21.45	6.12 a	4.45
		(NS)		(NS)*

† Herbicide treatments were applied 4 or 6 weeks after transplanting the crop plants.

‡ Numbers followed by the same letter are not significantly different according to the Duncan's Multiple Range Test at $P \leq 0.05$.

* NS refers to no significant difference.

Table 6. 6. Effect of 2,4-DB on broomrape growth following application of the herbicide to peanut foliage.

Treatment†	Rate	Infections‡ per plant	Shoot height	Shoot FW
	(kg/ha)		(cm)	(g)
Broomrape-infected control		2.40 a	2.82	0.50
2,4-DB (4 weeks)	0.1	0.14 b	0.24	0.43
2,4-DB (4 weeks)	0.2	0.24 b	0.11	0.48
2,4-DB (6 weeks)	0.1	1.17 ab	1.67	0.82
2,4-DB (6 weeks)	0.2	1.17 ab	0.00	0.52
			(NS)	(NS)*

† Herbicide treatments were applied 4 or 6 weeks after transplanting the crop plants.

‡ Numbers followed by the same letter are not significantly different according to the Duncan's Multiple Range Test at $P \leq 0.05$.

* NS refers to no significant difference.

6.3.2 Translocation and Metabolism of Glyphosate

In order to understand the nature of selective control of broomrape in crops by glyphosate, the translocation and metabolism of glyphosate was evaluated in tomato plants infected with *O. aegyptiaca*. Autoradiographs of broomrape-infected tomato plants showed that the radiolabel from ^{14}C-glyphosate applied on tomato leaves was translocated to all parts of the host plant and particularly to broomrape, where it appeared to accumulate in the shoot meristem within 3 days of treatment (Figure 6.1). Autoradiographs of broomrape-infected tomato plants treated with ^{14}C-glyphosate for 7 days showed a similar distribution of the radiolabel (Figures 6.2 and 6.3) to that observed in plants treated with the herbicide for 3 days. However, when glyphosate was applied to tomato plants scarcely infected by broomrape (Figure 6.2), more radiolabel was translocated to the meristematic region of the crop plants than in tomato plants that supported more vigorously growing broomrape plants. This may be because broomrape acts as a much stronger sink for nutrients and water from the host than the apical meristem of the host plant. Therefore, it appears that the timing of glyphosate application on host plants in relation to the stage of development of broomrape may be an important factor in obtaining selective control of this parasitic weed in crops. Jacobsohn and Kelman (14) have reported on the advantages of proper timing of glyphosate application for broomrape control in carrot (*Daucus carota* L.) and celery (*Apium graveolense* L.).

Quantitative estimation of the radiolabel in various parts of the host plants and in broomrape shoots (on the basis of total radioactivity present per gram dry weight of broomrape shoot) by liquid scintillation spectrometry showed that only a fraction (less than 5%) of the radioactivity applied on tomato plants moved out of the treated leaves (Table 6.7). Radioactivity translocated from the treated tomato leaves showed greater accumulation in broomrape shoots than in the apical meristem of the host. Accumulation of radioactivity in broomrape shoots did not increase with the time of treatment on the host leaves from three to seven days (Table 6.7), thus indicating that most of the glyphosate translocation to

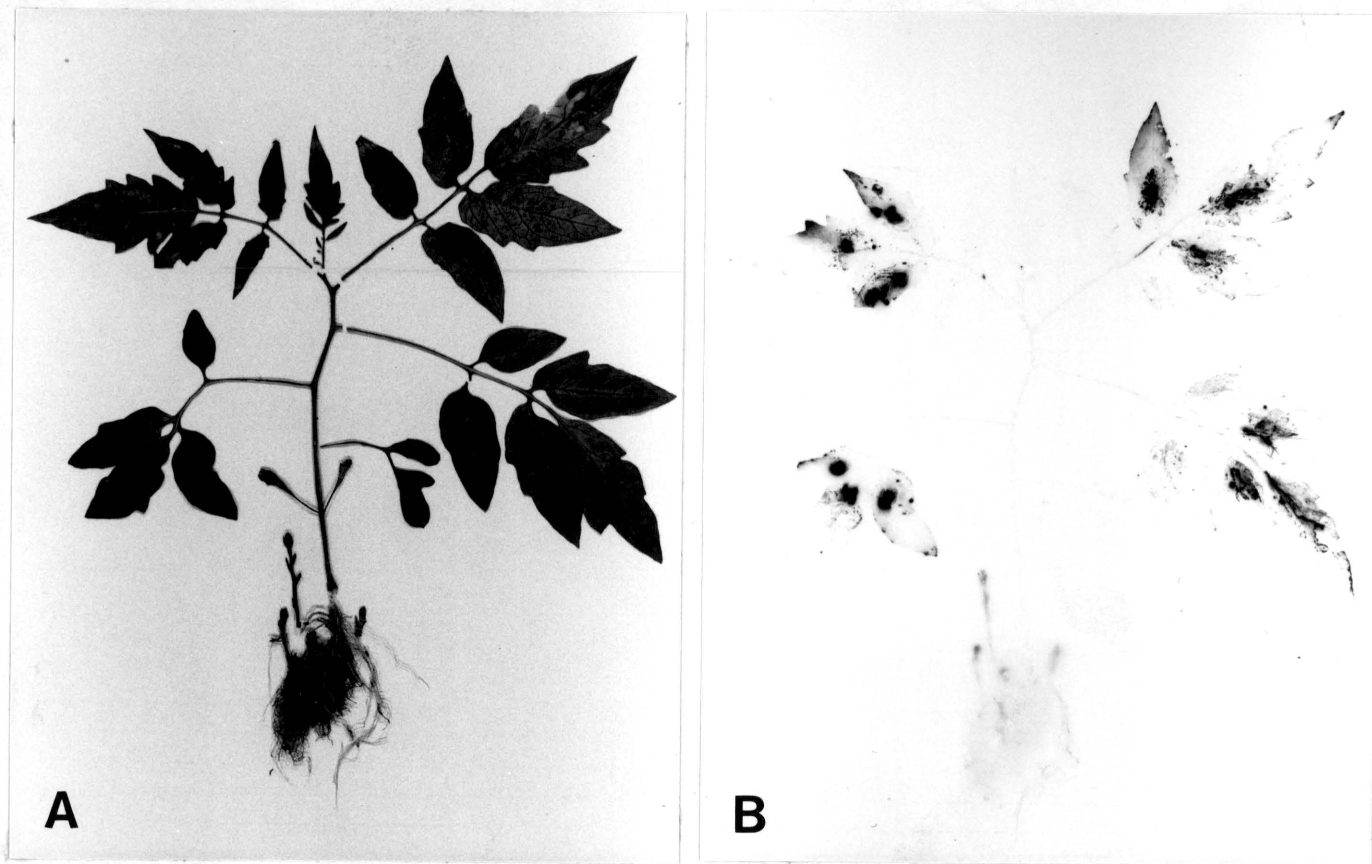

Figure 6. 1. (A) Dry mount and (B) autoradiogram of tomato (cv. Rutgers) plant 3 days after treatment with ^{14}C-glyphosate.

Figure 6. 2. (A) Dry mount and (B) autoradiogram of a tomato plant infected with broomrape at an early stage of parasite development 7 days after treatment with ^{14}C-glyphosate. (Note the distribution of radiolabel to the apical meristem of the host plant).

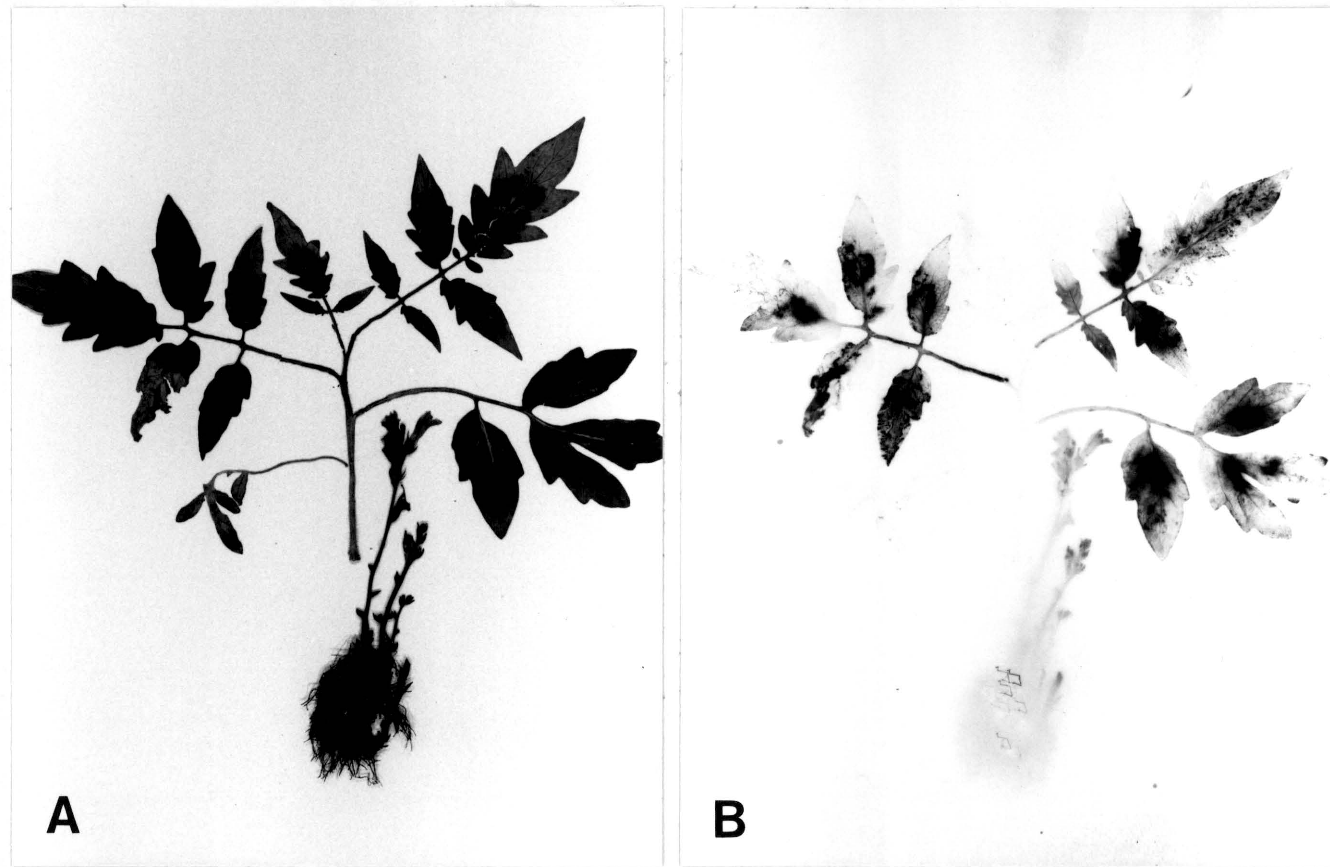

Figure 6. 3. (A) Dry mount and (B) autoradiogram of tomato (cv. Rutgers) plant 7 days after treatment with ^{14}C-glyphosate.

Table 6. 7. Distribution of ^{14}C-glyphosate in 'Rutgers' tomato plants infected with broomrape.

Plant part	After 3 days		After 7 days	
	Total DPM† $\times 10^3$	% of total‡ ^{14}C applied	Total DPM† $\times 10^3$	% of total‡ ^{14}C applied
Treated leaves	261.0	47.50	311.0	56.60
Apical meristem	4.0	0.73	1.1	0.20
Shoot (without treated leaves)	4.5	0.82	1.3	0.24
Roots	5.7	1.04	9.8	1.78
Broomrape shoots	8.2	1.50	8.6	1.57
	(61.5)		(58.6)	

† Total DPM refers to disintegrations per minute present in the plant part analyzed. Numbers in parenthesis are DPM/g dry broomrape tissue.

‡ Total radioactivity applied on each plant was 0.25 µCi, which was equivalent to 550,000 DPM.

broomrape shoots had occurred within 3 days of the herbicide application. Photoassimilates from tomato plants are readily translocated to the attached *O. ramosa* L. plants (30). The above results show that ^{14}C-glyphosate behaves in a manner similar to that of photoassimilates in a host-broomrape (tomato-*O. aegyptiaca*) system and that broomrape acts as a strong sink and competes directly with the apical meristem of the host for glyphosate and most probably photoassimilates.

Thin layer chromatography of extracts obtained from various parts of the host and the broomrape plants showed that the radioactivity present in either tomato or broomrape was in the form of the intact glyphosate molecule (Figure 6.4). The Rf value of radioactivity present in broomrape and tomato shoots ranged between 0.2 and 0.3. Sprankle et al. (35) have reported that the Rf value of glyphosate ranged between 0.28 and 0.32 and that of the major metabolite of glyphosate, aminomethyl phosphonic acid, ranged from 0.41 to 0.44. The other metabolites of glyphosate, glycine and sarcosine have much higher Rf values than aminomethyl phosphonic acid. The procedure and the materials (TLC plates and the solvent system for developing the plates) used in the experiment were taken from Sprankle et al. (35).

Glyphosate has been observed to be quite stable in many plant species (11, 33). Devine and Bandeen (8) have reported that as much as 80% of applied glyphosate could be detected in plants treated in the autumn and analyzed in the following spring, and Coupland (3) observed that only a small amount of glyphosate was lost as CO_2 from the rhizomes of quackgrass [*Agropyron repens* (L.) Beauv.] growing under warm conditions. The stability of glyphosate is needed with respect to broomrape control, however, persistence of the herbicide is not desirable in the host plants, many of which are important sources of food.

6.3.3 Effect of Glyphosate on Seed Germination of Broomrape

It is now well known that broomrape seeds germinate only when they come in contact with a chemical stimulant present in root exudates of host and some nonhost plants.

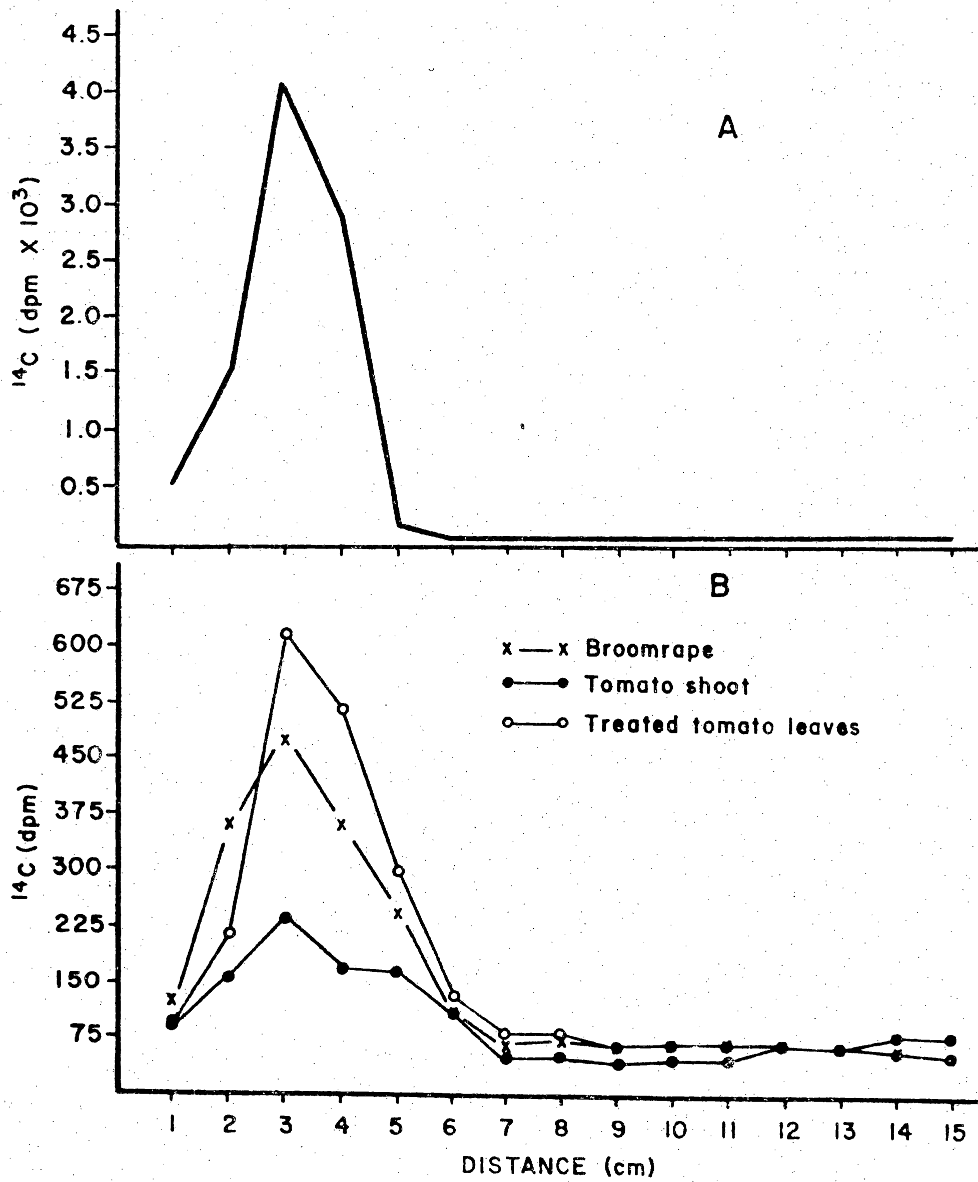

Figure 6. 4. Thin layer chromatographic analysis of (A) ^{14}C-glyphosate standard and (B) ^{14}C-glyphosate in extracts of broomrape, tomato shoots, and treated tomato leaves.

Considering the fact that small quantities of foliarly applied glyphosate may also be exuded through the roots of crop plants into the rhizosphere, the effect of low concentrations of this herbicide was evaluated on the germination of broomrape seeds.

A surprisingly high spontaneous germination (about 17%) of broomrape (*O. aegyptiaca*) seeds incubated in distilled water was observed in this experiment (Table 6.8). Seeds preconditioned in glyphosate solutions followed by postconditioning in distilled water showed a significant increase in germination at the two lowest concentrations of the herbicide used. At higher concentrations of glyphosate (1 to 10 mM) germination was gradually inhibited, with no germination occurring at the highest concentration of the herbicide. Broomrape seeds preconditioned in water and then postconditioned in glyphosate at the lowest concentration also showed a significant increase in germination over the distilled water control. However, at higher (0.1 to 10 mM) glyphosate concentrations, there was a gradual decrease in germination with an increase in herbicide concentration. No germination of broomrape seeds was observed when they were treated with 10 mM glyphosate after preconditioning in distilled water. Percent germination at equimolar concentration was significantly lower when broomrape seeds were treated with glyphosate during postconditioning than when they were treated with the herbicide during preconditioning, thus indicating that preconditioned broomrape seeds were more sensitive to glyphosate than nonpreconditioned seeds.

Broomrape seeds stimulated to germinate with GR 7 applied during postconditioning showed almost three times more germination than seeds incubated in distilled water but not treated with a germination stimulant (Table 6.9). Seeds preconditioned in glyphosate solution and then stimulated to germinate with GR 7 showed a significant inhibition of germination at a glyphosate concentration of 1 mM or higher. Treatment of water-preconditioned seeds with glyphosate followed by application of GR 7 one day after treatment with glyphosate resulted in a significantly lower germination than that of water-preconditioned broomrape seeds treated with GR 7 alone. As observed with nonstimulated broomrape seeds (Table 6.8), treatment of seeds with glyphosate during postconditioning was much more effective in

Table 6. 8. Effect of glyphosate on germination of ***O. aegyptiaca*** seeds.

Preconditioning treatment	Postconditioning treatment	germination†
		(%)
Water	Water	17.26
Glyphosate 0.01 mM	Water	38.33
Glyphosate 0.10 mM	Water	32.45
Glyphosate 1.00 mM	Water	15.10
Glyphosate 10.0 mM	Water	0.00
Water	Glyphosate (0.01 mM)	27.28
Water	Glyphosate (0.10 mM)	17.65
Water	Glyphosate (1.00 mM)	1.23
Water	Glyphosate (10.0 mM)	0.00
	LSD (0.05)	8.63

† Numbers are means of four replicates. Each replication consisted of about 100 seeds.

Table 6. 9. Effect of glyphosate on germination stimulation of ***O. aegyptiaca*** seeds by GR 7.

Preconditioning treatment	Postconditioning* treatment	germination
		(%)
Water	GR 7 5 µM	47.38
Glyphosate 0.01 mM	GR 7 5 µM	42.55
Glyphosate 0.10 mM	GR 7 5 µM	43.50
Glyphosate 1.00 mM	GR 7 5 µM	27.18
Glyphosate 10.0 mM	GR 7 5 µM	0.00
Water	Glyphosate (0.01 mM) + GR 7 5 µM	21.08
Water	Glyphosate (0.10 mM) + GR 7 5 µM	34.58
Water	Glyphosate (1.00 mM) + GR 7 5 µM	16.00
Water	Glyphosate (10.0 mM) + GR 7 5 µM	1.68
	LSD (0.05)	8.63

* During postconditioning treatment, glyphosate and GR 7 were applied sequentially with a difference of 1 day.

inhibiting germination of stimulated broomrape seeds than treatment with the herbicide during preconditioning.

Few reports exist in the literature on the effect of glyphosate on broomrape seed germination. However, the effect of several other herbicides has been investigated by various researchers. Kasasian and Parker (18) evaluated two hundred and thirty five chemicals for their effect on *O. aegyptiaca* seed germination. Oryzalin, dichlobenil, chlorthiamid, nitralin, and chloramben were regarded as promising for the preemergence control of broomrape (see Table 6.10 for chemical names of herbicides). Diphenamid was observed to affect both germination (25) and the radicle length of germinating broomrape seeds (28). Trifluralin at 10 to 100 ppm also reduced the radicle length of germinating broomrape seeds. Much higher concentrations (1000 to 3000 ppm) of the herbicide were required to inhibit broomrape seed germination (28).

Coupland and Caseley (4) reported that appreciable amounts of ^{14}C-glyphosate were exuded from intact roots of quackgrass into the surrounding solution. It has also been observed that glyphosate exuded into the surrounding medium could be absorbed by the roots of adjacent plants and affect their growth (26). The possible effects of glyphosate exuded from plants on soil microorganisms have also been postulated (4). The above results indicate that low concentrations of glyphosate that may be exuded by crop plants treated with the herbicide can inhibit germination of broomrape seeds present in the immediate vicinity of host roots.

Table 6. 10. Common and chemical names of compounds included in the discussion.

Common name	*Chemical name*
Chloramben	3-amino-2,5-dichlorobenzoic acid.
Dichlobenil	2,6-dichlorobenzonitrile
Diphenamid	N,N-dimethyl-2,2-diphenylacetamide
Nitralin	4-(methylsulfonyl)-2,6-dinitro-N-N-dipropylaniline
Oryzalin	3,5-dinitro-N^4,N^4-dipropylsulfanilamide
Trifluralin	α,α,α-trifluoro-2,6-dinitro-N,N- dipropyl-p-toluidine

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7.0 INFLUENCE OF VARIOUS NUTRIENTS AND STRIGOL ANALOGS ON GERMINATION AND PARASITISM OF BROOMRAPE

7.1 INTRODUCTION

Broomrapes (*Orobanche* spp.) occur most commonly in areas with hot and dry climates such as those of the middle eastern countries, the Mediterranean region, and Southern Europe. These areas frequently lack moisture and some areas, particularly in the middle eastern countries, are of poor soil fertility (1, 10). Low fertility is thought to be an important factor in witchweed (*Striga* spp.) (9) and broomrape (1, 10) infestation.

Increases in crop vigor and competition for nutrients and water following fertilization of the soil both reduce broomrape parasitism. Farmers in Jordan have claimed that the addition of manure to soil can reduce the infestation of broomrape in their fields (1). High levels of nitrogen alone reduced the yield of the crops. When potassium and phosphorus were

added with nitrogen, however, broomrape infestation was reduced drastically and crop yields were increased (2). Manure and other nitrogenous fertilizers have been reported to reduce crop damage due to the parasite (3, 11, 13, 15), but others have attributed the beneficial effects of nitrogen to improved crop performance and tolerance to attack (7, 16).

The objectives of this study were to determine the effect of various nutrients on parasitism of tomato (*Lycopersicon esculentum* Mill.) plants by *O. aegyptiaca* in the greenhouse and to investigate the influence of these nutrients on stimulation of *O. aegyptiaca* seed germination by synthetic growth regulators in the laboratory.

7.2 MATERIALS AND METHODS

7.2.1 Greenhouse Experiments

The procedure used in this experiment was similar to that used for the potting media experiment. Tomato (var. Rutgers) plants at the two-true-leaf stage were transplanted into 15-cm diameter plastic pots containing a potting medium. The potting medium consisted of equal volumes of clay loam, sand, and Weblite. Tomato plants were inoculated at the time of transplanting by dispersing about 5 mg of broomrape seeds (same seed lot as that used in the potting media experiment) directly on the roots of the crop plants. Solutions of ammonium nitrate at 2.9 g/pot (equivalent to 1 g N/kg of potting medium) or potassium phosphate at 1.4 g/pot (equivalent to 1 g PO_4/kg of potting medium) were applied either alone or in combination to tomato plants 1 week after transplanting. The pH of the nutrient solutions was adjusted to 6.5 using 0.01 M Na_2HPO_4/NaH_2PO_4 buffer. Only minute quantities of this buffer were required to adjust the pH of the nutrient solutions. Plants were harvested 10 weeks after transplanting,

and observations on tomato shoot height and root and shoot fresh weights (FW) and broomrape number of infections, shoot height above soil surface, and shoot fresh weight were recorded. The treatments were replicated six times, and the data were analyzed statistically using the least significant difference (LSD) test at the 5% level of significance. The experiment was repeated once.

7.2.2 Laboratory Experiments

As described earlier, the germination process of broomrape can be divided into two phases. The first is a preconditioning phase during which the seeds become receptive to a germination stimulant after being incubated for about 10 days under suitable temperature and moisture. The second is the stimulant treatment phase during which the seeds produce a germ-tube-like radicle. Seeds that produce a radicle are regarded as germinated.

The standard germination procedure as described earlier was used to study the effect of various nutrients on germination of broomrape seeds. *O. aegyptiaca* seeds surface-sterilized in Sporicidin were preconditioned on GFFP disks placed on a single sheet of Whatman 5 filter paper in 9-cm petri dishes. Each petri dish was given 3 ml of distilled water or any one of the test solutions containing various nutrients. The pH of the test solutions was adjusted to 6.5 using 0.01 M Na_2HPO_4/NaH_2PO_4 buffer. The petri dishes were sealed with a strip of parafilm to prevent loss of moisture during preconditioning; placed in a plastic container which was wrapped in aluminum foil to exclude all light. The seeds were incubated at 22 to 25°C for 10 days.

After the preconditioning period, broomrape seeds were treated with a germination stimulant. The GFFP disks containing the broomrape seeds were removed from the petri dishes and placed on new GFFP disks each soaked with 50 µl solution of the synthetic germination stimulant, GR 7 or GR 24. The control and the germination stimulant solutions

contained 0.1% DMSO. Treatment of broomrape seeds with the germination stimulants was carried out under 'safe' green light and the treated seeds were incubated in the dark at 22 to 25°C. Each petri dish contained four or eight GFFP disks with the seeds and represented one replicate. There were four or five replications for each treatment. Germination was counted using a binocular microscope 1 week after treatment with the germination stimulants. The results were analyzed using the F-test and the differences in means separated using the least significant difference (LSD) test at the 5% level of significance.

7.3 RESULTS AND DISCUSSION

7.3.1 Effect of Nutrients on Parasitism of Tomato Plants by Broomrape

In order to test the effect of various nutrients on broomrape parasitism, potting medium A consisting of equal volumes of clay loam, sand and Weblite (expanded shale) was used in these experiments. Mean number of broomrape infections on tomato plants in the control pots was 1.5. Parasitism by broomrape resulted in smaller shoot height and fresh weight of tomato plants than nonparasitized tomato plants (Table 7.1); however, these differences were not statistically significant. Application of ammonium nitrate at 2.9 g/pot alone or in combination with potassium phosphate at 1.4 g/pot completely inhibited broomrape parasitism and enhanced growth of tomato plants (Table 7.2). Application of potassium phosphate alone, although enhancing growth of tomato plants as compared to nonfertilized controls, did not reduce parasitism by broomrape. In fact, application of potassium phosphate resulted in significantly higher fresh weight of broomrape shoots as compared to the nonfertilized broomrape control.

Table 7. 1. Effect of various nutrients on growth of ***O. aegyptiaca*** parasitizing tomato plants.

Treatment†	Rate	Infections per plant	Shoot‡ height	Shoot F.W.
	(g/pot)		(cm)	(g)
Broomrape-infected control		1.5	5.7	0.5
Ammonium Nitrate	2.9	0.0	0.0	0.0
Potassium phosphate	1.4	1.3	3.8	2.1
Ammonium Nitrate + Potassium phospate	2.9 + 1.4	0.0	0.0	0.0
LSD (0.05)		0.6	2.1	1.2

† Treatments with nutrient solutions were applied one week after transplanting and the plants were harvested eight weeks after transplanting.

‡ Shoot height of broomrape is height above the surface of the potting medium.

Ammonium nitrate when applied alone inhibited parasitism of tomato plants by *O. aegyptiaca* but did not enhance the growth of tomato plants relative to nonfertilized controls.

Table 7. 2. Effect of various nutrients on growth of tomato plants.

Treatment†	Rate	Plant height	Shoot F.W.	Root F.W.
	(g/pot)	(cm)	(g)	(g)
Noninoculated control		18.4	2.1	1.1
Broomrape infected control		13.2	1.1	1.0
Ammonium Nitrate	2.9	16.0	1.3	0.5
Potassium phosphate	1.4	30.7	8.7	3.0
Ammonium Nitrate + Potassium phospate	2.9 + 1.4	56.5	49.0	7.6
LSD (0.05)		6.5	7.2	1.2

† Treatment with nutrient solutions were applied 1 week after transplanting and the plants were harvested 8 weeks after transplanting.

This may be due to nitrogen toxicity, especially to the roots, which showed lower fresh weight than the controls. Inhibition of broomrape parasitism by ammonium nitrate, however, does not seem to occur due to reduced root mass of tomato plants since fertilization by ammonium nitrate in combination with potassium phosphate increased the root mass considerably but did not allow parasitism by *O. aegyptiaca*. On the other hand, fertilization by potassium phosphate enhanced growth of tomato plants as compared to nonfertilized controls but did not inhibit parasitism by *O. aegyptiaca*. Thus diminished infection of tomato plants by *O. aegyptiaca* after fertilization may not be due to increased vigor of the host as has been noted by Ernst (8) working with *O. ramosa* L. parasitism on tobacco (*Nicotiana tabacum* L.) plants.

These results confirm reports in the literature that nitrogen application reduces parasitism of crop plants by broomrape. Abu-Irmaileh (1, 2) reported that application of ammonium sulfate alone or in combination with phosphorus and potassium drastically reduced broomrape parasitism on tomato plants. Similar results have been reported on the parasitism of witchweed (*Striga asiatica*. (L.) Kuntze) on corn (*Zea Mays* L.) (9). The beneficial effect of ammonia fertilizer may be a direct interaction with the metabolism of broomrape. An increased ammonium supply generally reduces the uptake of potassium (14) and may affect the osmotic balance of broomrape. An increase in the osmotic potential of the host cell sap can prevent the translocation of organic substances to the parasite via host phloem, which may ultimately result in reduced broomrape parasitism (6).

7.3.2 Effect of Various Nutrients on Germination of Broomrape Seeds

In order to investigate further the mechanism by which fertilization of the potting medium reduces broomrape parasitism on tomato plants, the effect of various nutrients was investigated on the germination of broomrape seeds in the laboratory. Preconditioning of broomrape seeds in various nutrients showed considerable differences in germination

percentage when stimulated to germinate with 1 ppm GR 24 (Figure 7.1). Seeds preconditioned in NaCl control at 1 or 10 mM concentration showed about 37% germination after treatment with GR 24. Very low (3% or lower) spontaneous germination was observed in any nutrient solution. Preconditioning of seeds in ammonium nitrate alone or in combination with potassium phosphate gave equal or higher percent germination in comparison to NaCl preconditioned seeds. Preconditioning in phosphoric acid, potassium sulfate or potassium phosphate alone gave significantly lower germination than NaCl preconditioned seeds.

O. aegyptiaca seeds were preconditioned in sodium nitrate, ammonium sulfate or urea to determine if nitrate and ammonium forms of nitrogen had similar effects on broomrape seed germination. Preconditioning in ammonium sulfate or urea resulted in significantly lower germination than preconditioning in the NaCl control. Germination of seeds preconditioned in sodium nitrate was not significantly different from that of seeds preconditioned in the NaCl control (Figure 7.2).

There are few reports in the literature on the effect of nutrients on germination of *Orobanche* seeds. Brown and Edwards (5), however, have reported that thiourea and allylthiourea could promote germination of *Striga* seeds in the absence of a stimulant from the host. Later Pesch and Pieterse (12) reported that urea and ammonium sulfate markedly decreased germination of *Striga* seeds. Pieterse and co-workers (cited in 4) observed that ammonium sulfate and urea reduced, whereas sodium nitrate had no effect on *O. crenata* Forsk. germination. Observations with *O. aegyptiaca* confirm these results. It is interesting to note that while nitrogen fertilization inhibits broomrape parasitism on tomato plants, preconditioning of broomrape seeds in nitrogen-containing solutions markedly enhances their germination *in vitro*. It may be concluded, therefore, that inhibition of broomrape parasitism on crop plants in the presence of nitrogen fertilizers is not due to an effect of the nutrient on broomrape seed germination but is more due to an effect on the physiology of the host plants. It has also been suggested that high nitrogen application can result in an osmotic injury to the germinating tube of broomrape seeds and thus affect the attachment of the parasite to host roots (2).

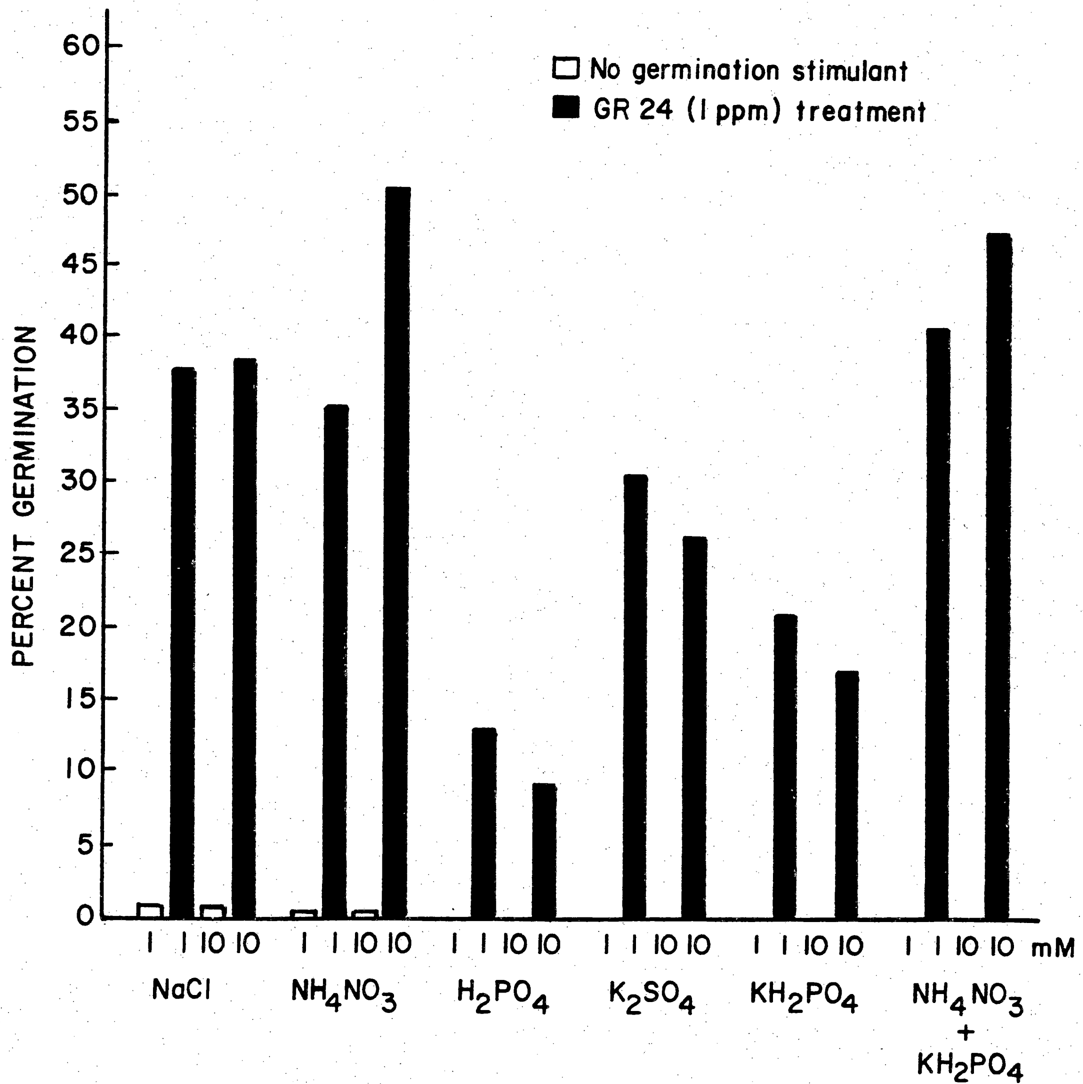

Figure 7. 1. Effect of various nutrients applied during preconditioning on germination of ***O. aegyptiaca*** seeds.

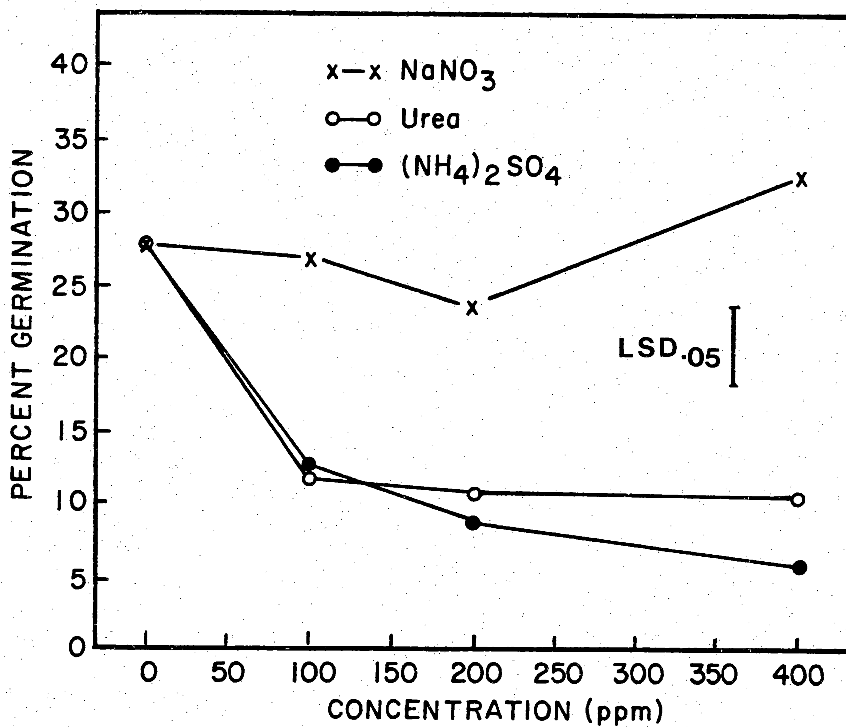

Figure 7. 2. Effect of nitrogenous compounds on germination of ***O. aegyptiaca*** seeds.

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8.0 SUMMARY AND CONCLUSIONS

The main objectives of this research were to determine the potential of three of the most devastating species of broomrape to parasitize some major broadleaf crops in the United States and to test an integrated approach for broomrape control in crops. This approach included investigating methods for reducing broomrape seed populations in the soil by inducing 'suicidal germination', preventing the production of new seeds by selectively controlling/suppressing broomrape after its attachment to host roots by means of systemic herbicides, and by proper fertilization of the soil to reduce broomrape parasitism on host plants.

All three species of broomrape, *O. aegyptiaca* Pers., *O. ramosa* L. and *O. crenata* Forsk., showed potential to parasitize one or more of the five major broadleaf crops, tomato (*Lycopersicon esculentum* Mill.), tobacco (*Nicotiana tabacum* L.), alfalfa (*Medicago sativa* L.), peanut (*Arachis hypogaea* L.), and soybean (*Glycine max* L.). *O. aegyptiaca* showed a wider host range than *O. ramosa* or *O. crenata* by parasitizing crops belonging to both Solanaceae and Fabaceae families. *O. ramosa* was restricted mainly to Solanaceae, whereas *O. crenata* parasitized crops belonging to Fabaceae. Peanut was most susceptible to broomrape being parasitized by all three broomrape species. Soybean was most resistant to parasitism by broomrape. Only one plant each of *O. crenata* and *O. ramosa* parasitized soybean plants.

Two synthetic analogs of strigol, GR 7 and GR 24, showed excellent potential to induce 'suicidal germination' of broomrape seeds in the laboratory. Ethylene, a very effective germination stimulant of *Striga* spp., was only moderately effective in inducing germination of broomrape seeds. Gibberellic acid was more effective than ethephon, but significantly less effective than the strigol analogs in inducing broomrape seed germination. Ancymidol, an inhibitor of endogenous gibberellin synthesis, was inhibitory to broomrape seed germination. The inhibitory action of ancymidol was more pronounced when it was applied to preconditioned than to nonpreconditioned broomrape seeds. Application of GR 24 to broomrape seeds treated with ancymidol at 0.1 mM or higher did not result in a significant stimulation of germination. However, when gibberellic acid was applied to broomrape seeds treated with ancymidol and GR 24, germination equivalent to that obtained after treatment of seeds with GR 24 alone was observed. These results indicated that the strigol analogs induce germination of broomrape seeds by triggering endogenous gibberellin synthesis, which was inhibited in the presence of ancymidol.

Glyphosate at 75 g/ha applied to tomato shoots was effective in controlling/suppressing the growth of broomrape after its attachment to host roots, but caused injury to the host plants. Single or multiple applications of glyphosate at 37.5 g/ha or lower proved ineffective in controlling broomrape in tomato. 2,4-DB at 0.1 kg/ha was effective in reducing the number of broomrape infections on peanut.

Application of ^{14}C-glyphosate on tomato leaves resulted in significant translocation of the radioactivity to all parts of the host plant and to broomrape shoots. More radiolabel was translocated to the apical meristem of tomato plants scarcely infected by broomrape than to the apical meristem of tomato plants severely infected by the parasite. In severely infected tomato plants, more radiolabel appeared to be translocated to broomrape shoots than to the apical meristem of the host plant. No metabolites of glyphosate were detected in any part of the host or the broomrape. These results indicate that glyphosate could be used for selective control of broomrape in crops. However, the timing of glyphosate application to crop plants may be an important criterion in preventing injury to the host plants from the herbicide.

Glyphosate at concentrations of 10 mM or higher inhibited germination of broomrape seeds in the laboratory, indicating that low concentrations of glyphosate that may be exuded by crop plants treated with the herbicide can possibly inhibit germination of broomrape seeds present in the immediate vicinity of the host roots.

Fertilization of the potting medium with nitrogenous compounds inhibited parasitism of tomato plants by broomrape. Ammonium nitrate applied alone or in combination with potassium phosphate did not affect the germination of broomrape seeds in the laboratory. These results indicated that inhibition of broomrape parasitism on crop plants in the presence of nitrogen fertilizers, especially nitrate, was not due to an effect of the nutrient on broomrape germination, but was probably due to an effect on the physiology of the host plant.

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